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CYTOLOGY AND MORPHOLOGY OF SOME HYBRIDS OF AEGILOPS X SECALE

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CYTOLOGY AND MORPHOLOGY OF SOME HYBRIDS OF AEGILOPS X SECALE

ABSTRACT

Twenty-four species of Aegilops including diploids, tetraploids and hexaploids were crossed with Secale cereale and the hybrids were subjected to a morphological and cytological study of intergeneric relationships. Twelve crosses were successful and amphidiploids were obtained from six of the hybrids. The crossability of Aegilops tended to increase with the chromosome number but species in a ploidy group differed considerably. Crosses involving all of the tetraploids and almost all of the hexaploid species readily produced mature hybrids, but only two were obtained from ten crosses with diploids.

Marked genetic differences between the diploid Aegilops species Ae. comosa (M genome) and Ae. squarrosa (D genome) were indicated by great differences in the proportion of morphological characters studied wherein the hybrids resembled their Aegilops parent. Such differences were not noted between the tetraploid, Ae. cylindrica (CD) and hexaploid Ae. crassa (DDM^{cr}). It was inferred that the C, M^{cr} and M genomes are similar but differ markedly from the D genome in degree of dominance in the hybrids.

The chromosomes of the species studied cytologically (Ae. squarrosa, Ae. comosa, Ae. cylindrica and hexaploid Ae. crassa) were found to be readily distinguishable from those of S. cereale in both the mitotic and meiotic divisions of the hybrids. Aegilops chromosomes paired with Secale chromosomes in all of the hybrids with varying frequencies. The heteromorphic bivalent frequency per cell in the hybrid involving Ae. squarrosa was 0.7,

Ae. comosa, 0.34 and Ae. cylindrica, 0.48. Autosyndesis increased markedly with increased ploidy, the hybrids involving diploids having 12, the tetraploid 90.5 and the hexaploid 93 percent of total bivalents formed by autosyndesis. In all four hybrids the potential pairing frequency as determined by Gaul and Munzner's formula was greater than the observed pairing frequency. The experimental results indicate that (1) the chromosomes of Aegilops and Secale that pair in the hybrids possess more than a residual homology; (2) the lengths of homologous regions are of sufficient magnitude as to permit prophase pairing and chiasma formation; (3) a genetic mechanism that reduces such pairing and chiasma formation, despite the presence of such homologies, is present in these hybrids.

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INTRODUCTION

Morphologically and karyologically, Aegilops L. is one of the most diverse genera in the tribe Hordeae. Cytotaxonomic studies have revealed a euploid series comprising 9 diploids, 9 tetraploids and 3 hexaploids with a basic number of 7. The knowledge that one of these diploids, Ae. squarrosa, participated in the formation of hexaploid wheat has stimulated widespread interest in the study of genome relationships among the species. More recently, the evidence that Ae. speltoides contains morphological and cytological characteristics which are common to wheat, thereby identifying a second species of this group involved in the phylogeny of wheat, has added further impetus to investigations seeking to interpret the affinities within this diverse genus.

Although interspecific and intergeneric hybrids involving Aegilops species have been studied since 1854, it is only in the past 30 years that cytological studies have achieved any prominence in this field. The method of genome analysis originated by Kihara has been applied to many cytological studies in this genus and has lead to a clearer understanding of the relationships between the species. However, in most cytological studies of interspecific or intergeneric hybrids, the inability to identify the chromosomes from the two parents during meiosis in the hybrid has made an accurate determination of genome relationships difficult and it was only possible to speculate whether the observed pairing was autosyndetic or allosyndetic.

Because the chromosomes of Secale are larger than those of Aegilops both in the parents and hybrids, it is possible to determine rather accurately the nature of pairing in the hybrid at meiosis. Thus heteromorphic bivalents indicate allosyndetic pairing and homomorphic bivalents, autosyndetic pairing. A study of this kind provides a quantitative analysis of chromosome pairing which might be used in clarifying the intergeneric relationships of Aegilops

and Secale. Furthermore a comparative morphological study of the parents and hybrids might provide additional information regarding those relationships. Following this approach, therefore, this study is based on the hybridization of 24 species of Aegilops with Secale and a morphological and cytological study of several of their hybrids.

LITERATURE REVIEW

The cytogenetic studies carried out with Aegilops and Secale were performed in the 1930's, during a period in which the cytological delineation of the chromosomes in the various species of Aegilops and Secale was not accurately known. Many studies in other genera performed since have, however, indicated that studies of chromosome-size differences of the order that exist between Aegilops and Secale augmented with studies of morphological differences in karyotype, may serve to provide a very accurate means of identifying parental chromosomes, and thus to distinguish allosyndetic from autosyndetic pairing in the hybrids.

The most extensive of the early studies in hybridization between Aegilops and Secale was made by Oehler (1934), who crossed 19 species of Aegilops with a variety of summer, winter, self- and cross-fertile types. The crosses involving diploid Aegilops were lowest in crossability (seed set), ranging from 0 to 16.2 percent and germination failed in all instances. The crosses involving tetraploid Aegilops showed a crossability ranging from 3.17 to 36.9 percent and a germination rate ranging from 0 to 100 percent. The only hexaploid crossed, Ae. triaristata, had a seed set of 21.3 percent and germination rate of 60.6 percent. Hybrids were established from eight of the tetraploids and the hexaploid, and morphological descriptions were given of each. Cytological studies were not reported.

Ae. triuncialis (2n=28) was used frequently by various workers because of its comparatively high crossability with rye (Tschermak 1913, Leighty et al 1926, Karpechenko and Sorokina 1929, von Berg 1931, Buchinger 1933, Kagawa and Chizaki 1934, and Aase 1935). Of these, von Berg, Karpechenko and Sorokina, and Kagawa and Chizaki included cytological analyses in their studies, and all concluded that the chromosomes of

Ae. triuncialis were not homologous with those of Secale. Karpechenko and Sorokina observed a mode of 5 bivalents in this hybrid. They were able to distinguish the 7 large chromosomes of Secale in late anaphase but could not determine whether they participated in allosyndetic or autosyndetic pairing.

Von Berg was the first to utilize the size-differential of chromosomes as an analytical tool in the hybrids of these genera. He found in Ae. triuncialis x S. cereale that 5 to 6 bivalents were formed by small chromosomes and that the 7 large ones of Secale remained as univalents. Although he believed that occasionally intergeneric pairing of chromosomes occurred, he did not provide any evidence of this.

Kagawa and Chizaki reported a mode of 6 bivalents in the same cross and, although they were also able to distinguish the large rye chromosomes from those Aegilops when they occurred as univalents, they had difficulty in determining their origin when associated as bivalents and trivalents. Another hybrid studied by these workers was Ae. cylindrica (2n=28) x S. cereale in which they observed 4 to 6 bivalents with a range of 2 to 7. They often observed one or more trivalents but again were unable to determine the parental origin of the chromosomes involved. The third hybrid that they analyzed was Ae. ovata (2n=28) x S. cereale in which they found 2 to 5 bivalents with an occasional trivalent. They encountered difficulty in distinguishing the parental chromosomes in this hybrid also, but concluded that allosyndesis as well as autosyndesis probably occurred.

A fact of historic interest is that Vavilov (1949) stated that the chromosomes of Aegilops and Secale are not homologous and therefore do not pair. Vavilov's authority, it appears, was Oehler who, in turn, had based his opinion on the work of von Berg and Karpechenko and Sorokina. Thus, on the basis of studies from a single intergeneric cross, a gener-

alization regarding chromosome homologies was made which encompassed the entire two genera. Since that time only one report of Aegilops-Secale hybridization has appeared (Zennyozi 1959).

The method of genome analysis involving parents differing in the size of chromosomes has been used successfully by several investigators with other species in the Gramineae. Stebbins and Pun (1953) analyzed the hybrid between Secale cereale and Agropyron intermedium, in which the Secale chromosomes appeared larger than those of Agropyron. They were able to determine that the chromosome pairing was largely autosyndetic among the Agropyron chromosomes, although one bivalent was formed between a Secale and an Agropyron chromosome.

Wagenaar (1959) studied the hybrid Hordeum jubatum x Secale cereale and was able to determine by observation of chromosome size that the majority of the bivalents were formed by autosyndesis of the smaller Hordeum chromosomes. He also reported two heteromorphic bivalents that were formed by intergeneric pairing.

Dewey (1961) found chromosomes of Agropyron desertorum to be about twice the size of those of Agropyron repens. His study of meiotic cells of the hybrid showed that the bivalents were formed exclusively by autosyndesis.

In a series of studies by Nakajima (1951, 1953, 1959a, 1959b) of hybrids of Haynaldia, Secale and Triticum persicum, no heteromorphic bivalents were observed in 2500 metaphase cells of the hybrid H. villosa x S. cereale (0-2 bivalents, with 81.3 percent of the cells showing no pairing). However, in a study of the hybrid S. fragile x H. villosa, his data indicate that 7.4 percent of the 0-3 bivalents present were heteromorphic. An unusual and most interesting aspect of this study is the partial fertility of the F_1 hybrids which the present study, in a subsequent section, relates to a genetic mechanism that induces pairing between homoeologous chromosomes. Eight of

the 11 hybrids showed fertility ranging from 2.7 to 5.9 percent. No information was given on the cytology of the second generation, so it is not known whether these were F_2 hybrids or first generation amphidiploids. Fertility of F_1 intergeneric hybrids, although most exceptional, was also reported by Rick in the cross between Lycopersicon esculentum and Solanum pennellii (1959).

An interesting hybrid is that of Paraixeris denticulata (2n=10) x Lactuca squarrosa (2n=18) reported by Ono (1943). The F_1 hybrids ranged in chromosome number from 10 to 14 with the majority having 12. In the 12-chromosome hybrids there were usually 2 small fragments which participated in pairing. Most of the meiotic cells of these formed 6 bivalents, many of them heteromorphic. The fragments paired with larger chromosomes and univalents and trivalents were formed frequently. Although fertility of these hybrids was low, several achenes were obtained for the second generation. Ono and Sato (1935) also described another hybrid between Paraixeris denticulata and Crepidiastrum platyphyllum, which regularly formed 5 heteromorphic bivalents and which was fertile also.

The occurrence of intergeneric pairing of chromosomes in the hybrids described in these reports indicates that such a phenomenon is widespread in these families. The ability to distinguish the chromosomes of the parents in the hybrids provides valuable information on the extent of inter- and intragenome homology of chromosomes and suggests that genetic relationships can be much closer than morphological comparisons would indicate.

The phenomenon of a size-differential between the chromosomes of different species of the same genus is not uncommon. Kostoff (1938) found heteromorphic bivalents in Nicotiana glauca x N. Langsdorfii, an indication that allosyndetic pairing occurred. He concluded that complete homology was not necessary for synapsis and crossing-over, and suggested that the homoeology

of heteromorphic chromosomes may be due to a translocation or, alternatively, a deletion. Hakansson (1934) observed a pronounced dissimilarity in chromosome size between two Godetia species, G. deflexa having 18 large chromosomes and G. Bottae, 18 small ones. Similarly, Phillips and Endrizzi (1960) reported a case in Gossypium wherein the chromosomes of G. arboreum are larger than those of G. raimondii. The hybrids in both instances maintained the size difference of chromosomes and permitted a quantitative analysis of pairing frequencies, a discussion of which is included in a subsequent section.

A hybrid which requires investigation of meiotic pairing is that of Pennisetum purpureum (Napier grass) x P. glaucum (cattail millet) reported by Burton (1944). The chromosomes of the latter are about twice as large as those of the former and this difference is present in the hybrid.

The occurrence of pairing by chromosomes of unequal size from species of the same genus strongly suggests that genetic differentiation does not always accompany chromosomal differentiation. It also suggests that genetic control of chromosome size may be a factor in the evolution of some species as discussed by Darlington (1937). According to the review by Chennaveeraiah (1960) no such marked differences appear to exist in those species of Aegilops studied. The author therefore believes that considérable reservation must be held with regard to the interpretation of pairing in interspecific Aegilops hybrids. For this reason, and because the author believes that considerably more valid information on the interspecific differences may be obtained from the study of intergeneric hybrids with Secale, the study of such hybrids has not been pursued in this work, and an extensive summary of the literature (see Chennaveeraiah 1960 for review) is not presented here.

Stebbins' (1951) discussion of phylogenetic reduction in chromosome size provides interesting material for studies of hybrids with heteromorphic

chromosomes. The chromosomes of Crepis neglecta and C. fuliginosa are different in size in both the parents and the hybrid. In the genus Muscari of the Liliaceae, M. tenuiflorum, M. caucasicum and M. monstrosum have smaller chromosomes than those M. longipes. In the tribe Cichorieae, the genus Taraxacum has larger chromosomes than those of Sonchus. The genus Dianthus of the Caryophyllaceae contains a species, D. armeria in which the annual has smaller chromosomes than the perennial. Similarly, Stebbins indicates that the two annual species, Phalaris canariensis and P. paradoxa have smaller chromosomes than the perennial species of Phalaris.

Since Stebbins' discussion, a number of other genera with marked interspecific differences in chromosome size have been reported. Sharma and Bhattacharjee (1957) found that the chromosomes of Sorghum purpureosericeum are about 5 times as long, and those of S. versicolor about twice as long, as those of other species in the genus. Tateoka (1960) cites Swallen's (1928) observation that the chromosomes of Melica are considerably larger than those of its close relative Schizachne, and Brown's (1951) observation that the chromosomes of Elyonurus tripsacoides and E. barbiculmis are much larger than other members of the Andropogoneae. According to Celarier (1957), Elyonurus argenteus has the largest chromosomes in the tribe and (1956) those of Andropogon distachyus are about twice as long as most other members.

An unusual instance of extreme difference in chromosome size between species of the genus Oxalis was described by Marks (1957). In most species of Oxalis, the chromosomes are small, ranging from 1 to 4 microns in length, but in Oxalis dispar the chromosomes range from 14 to 24 microns in length.

This review of reports on species containing unequal-sized chromosomes is not intended as a complete treatise on the subject but rather as a

means of emphasizing the possibilities of extending the analytical approach used in the present study to other tribes and families. It is only in this way that knowledge will be gained of the extent to which specific genetic mechanisms occur in flowering plants.

Riley and Chapman (1958) and Sears and Okamoto (1958) reported that in certain 20-chromosome nulli-haploids and in crosses between diploid and nullisomics of hexaploid wheat, chromosome pairing in the nulli-haploid and hybrid were very much greater than in polyhaploids and in similar crosses with hexaploid wheat. They attributed this to the absence of a chromosome in the B genome, identified by Sears and Okamoto as chromosome V, that carries a gene or genes controlling the pairing of homoeologous chromosomes. Thus in the presence of chromosome V or its long arm, on which the pairing restrictor occurs (Riley et al 1960), the chromosomes of the A, B, and D genomes are withheld from intergeneric pairing, despite the intergenome homologies known to exist. In crosses between aneuploid wheat and S. cereale, pairing in those hybrids containing chromosome V was very low, whereas in those lacking it autosyndetic pairing increased sharply and intergeneric pairing occurred as well. Thus the effect of the mechanism on this chromosome was not only directed against the pairing of homoeologous members in the wheat complement but also against intergeneric pairing. Riley et al. (1958) and Riley (1960) also suggested that Ae. speltoides contains a gene or genes that suppress the action of chromosome V. Thus in the hybrids between wheat and Ae. speltoides the same degree of pairing occurs, regardless of the presence or absence of chromosome V. Riley therefore concluded that the mechanism on chromosome V arose by mutation after incorporation of the chromosome in polyploid wheat.

These interpretations constitute one of the most important advances in plant cytogenetics of the decade. The knowledge that such

rigid genetic control of chromosome pairing is possible has a profound bearing on future cytotaxonomy and poses many questions regarding earlier conclusions in this field. For example, the extensive studies of wheat-rye hybrids, which generally showed a very low frequency of pairing, led to conclusions of non-homology and placed wheat and rye in well separated categories. Evidence that rye chromosomes can pair with those of wheat and other genera indicates that a modification of our concepts regarding chromosome homologies and classification of these genera are required.

A review of the numerous reports on wheat-rye hybridization is not considered essential to the present study inasmuch as these hybrids were all under similar genetic control regarding pairing frequencies. Furthermore, two excellent reviews of the subject have appeared within recent years (O'Mara 1953, Jain 1960) which provide more detailed information than is possible within the scope of the present study. For these reasons this review is limited to consideration of the intergeneric hybrids involving Secale as well as other genera that are related to the topics of this study.

MATERIALS AND METHODS

1. Parents

Seeds of the male parent, Secale cereale L. var. Prolific were obtained from local stocks. The Aegilops species used as females are listed in Table 1.

2. Crosses and embryo cultures

Crosses were made in 1957, 1958 and 1959. The spikes were emasculated and bagged, and pollinated 2 to 5 days later. The florets were checked periodically to observe the progress of seed formation.

Seeds with apparently normal endosperm development were allowed to reach maturity and were then removed and placed in moist petri dishes under aseptic conditions. If germination did not occur after 10 days the seeds were dissected and the embryos cultured in vitro. Seeds with abnormal endosperms were removed before maturity and the embryos cultured. The shortest post-fertilization period after which an embryo could be cultured successfully was 12 days.

To avoid contamination, cultures were made in a small dust-free room in which a cloth moistened in 2% Dettol solution was spread over the table. The scalpel and fine-pointed tweezers used in dissecting were kept sterile in 95% alcohol. The mature seeds were surface-sterilized in 10% calcium hypochlorite for 5 to 10 minutes and washed in 3 changes of distilled water. Although the immature seeds were not sterilized, contamination was a minor factor.

The seeds were dissected aseptically under a microscope and the embryos removed with a small platinum loop or with tweezers to slants of solid media in 6-inch, screw-cap test tubes. They were then transferred to 6-inch pots with a 1:1 mixture of soil and sand and placed in the greenhouse.

TABLE 1

SPECIES OF AEGILOPS CROSSED IN PROGRAM

Species	Chromosome number (2n)
Aegilops caudata L.	14
" umbellulata Zhuk.	14
" squarrosa L. var. typica Zhuk.	14
" comosa Sibth. et Sm.	14
" uniaristata Vis.	14
" mutica Boiss.	14
" speltoides Tausch var. ligustica (Savign.) Fiori	14
" bicornis (Forsk.) Jaub. et Sp.	14
" longissima Schweinf. et Muschl.	14
" sharonensis Eig.	14
" cylindrica Host ssp. pauciaristata Eig.	28
" ovata L.	28
" triaristata Willd. ssp. triaristata Eig.	28
" columnaris Zhuk.	28
" biuncialis Vis.	28
" variabilis Eig.	28
" triuncialis L. ssp. typica Zhuk.	28
" crassa Boiss. ssp. crassa Eig.	28
" ventricosa Tausch	28
" triaristata Willd. ssp. recta Zhuk.	42
" crassa Bois. ssp. Vavilovii Zhuk.	42
" juvenalis (Thell.) Eig.	42

The above species, with the exception of two, were obtained from Dr. R. C. McGinnis and Dr. B. C. Jenkins of the University of Manitoba, Winnipeg, Manitoba. Ae. squarrosa var. typica and Ae. cylindrica var. pauciaristata were obtained from Dr. E. B. Wagenaar, Canada Department of Agriculture, Ottawa.

The culture media varied in composition. Some embryos responded to Orchid Agar, a mixture of mineral salts, sucrose and agar. Others were cultured on media containing tomato juice, vitamins, casamino acids, yeast, gibberellic acid, indole acetic acid, kinetin and coconut milk added individually or in various combinations. The agar medium was autoclaved for 20 minutes at 15 lbs. pressure. The heat labile substances were sterilized by filtration with a Kreuger filter.

The seeds produced by the crosses could be classified into five types according to the stage of development of the endosperm and embryo:

(a) Endosperm fully formed, normal embryo.

(b) Endosperm fully formed, abnormal embryo.

(c) Endosperm fully formed, no embryo.

(d) No visible endosperm, all embryo.

(e) Degenerate or fluid endosperm, with an embryo as in a, b, or c.

The crosses between diploid Aegilops and Secale almost invariably produced seeds of type "e". On the other hand, the seeds from the tetraploid and hexaploid species varied from types "a" to "d" with the exception of Ae. ventricosa and tetraploid Ae. crassa which frequently produced seeds of type "e".

The hybrid embryos from the diploid crosses varied from nearly normal ones to globular masses of tissue. Those of Ae. squarrosa x S. cereale varied in this way. The undifferentiated globular types were cultured during the early part of the program but, because of a consistent lack of response to culturing, this procedure was discontinued and they were discarded. This is one of the reasons why in Tables 2 and 3 it appears that not all of the kernels were seeded. Some of them were discarded as abnormal and some were retained as reserve material. Occasionally

advanced embryos with well-developed coleoptiles and coleorhizas were encountered. These were always characterized by a curled scutellum if they were of hybrid origin, as confirmed later by cytological observations of the seedling. Thus it was possible to distinguish hybrid embryos from those produced by accidental self-pollination, because the latter usually had a regular scutellum.

The seeds from the crosses involving the tetraploid and hexaploid species frequently appeared to contain a normal embryo and endosperm. These seeds nevertheless often failed to germinate and it was necessary to culture the embryos if the supply of seed was scarce. In most cases, however, enough seeds were available to ensure the germination of a few without resorting to culturing. When cultured embryos did not germinate within 10 days, they were subjected to cold treatment at 3 - 5 degrees centigrade for 58 hrs. and subsequently returned to room temperature.

3. Colchicine treatments

After 5 to 10 tillers were removed for pollen-mother-cell analyses, the plants were either cloned or left intact, depending on the number of hybrids from each cross available for treatment. Clones were permitted 1 to 2 weeks in which to become re-established after cloning. When several of the tillers were at the 2- to 4-leaf stage, the hybrids were treated with colchicine at a concentration of either 0.1, 0.5, 0.35, or 0.25 per cent.

The stage of development of the growing point at the one-leaf stage is shown in Plate 1, Fig. 1 and at the 3- to 4-leaf stage in Fig. 2. At the 1- to 2-leaf stage the growing point elongates slightly but does not differentiate. Floral differentiation of the growing point begins at the 3-leaf stage for most of the Aegilops species. The most active period of cell division in the floral primordia therefore is at the 3- to 4-leaf

stage. Since one of the prerequisites for successful induction of polyploidy with colchicine is an actively dividing meristem (Eigsti and Dustin, 1955), this stage was chosen as the one most appropriate for treatment.

The colchicine solution was applied by the vacuum-infusion method (Gentcheff 1955) with some modifications. The top layer of soil covering the crown of the plant was removed and a plastic disk was placed around the plant to prevent excessive washing away of the soil during treatment (Plate 1, Fig. 3). The pot was then inverted and the plant placed in a jar of colchicine solution with the rim of the jar resting against the plastic disk (Fig. 4). The pot and jar were then placed in a large desiccator and the air was evacuated to a pressure of 350 mm. of mercury for 5 - 15 minutes (Fig. 5). After the reintroduction of air, the plant was either removed immediately or left for 10 - 15 minutes for further infiltration. It was then placed in a transparent, humid chamber overnight under an illumination of approximately 2,000-foot candles for 24 hours in the greenhouse.

4. Chromosome analyses

(a) Meiosis

Spikes were fixed in Carnoy's solution and cytological analyses were made of cells squashed in aceto-carmine. Records were kept of the number and derivation of univalents (viz. from Aegilops or Secale) and also the number and kind of chromosomal associations.

Gaul and Münzner's formula for asynaptic hybrids (1955) was applied to the data in order to determine the maximum number of chromosomes potentially capable of pairing, (p). Rod bivalents were scored as having one chiasma, ring bivalents and trivalents as two. The formula is $p = \frac{X^2 + X - B}{Z(2X-B)}$ where X is the total number of chiasmata, B the total number

PLATE I

Figure 1. Growing point of Ae. comosa at first leaf stage.

Figure 2. Growing point of Ae. comosa at third to fourth leaf stage.

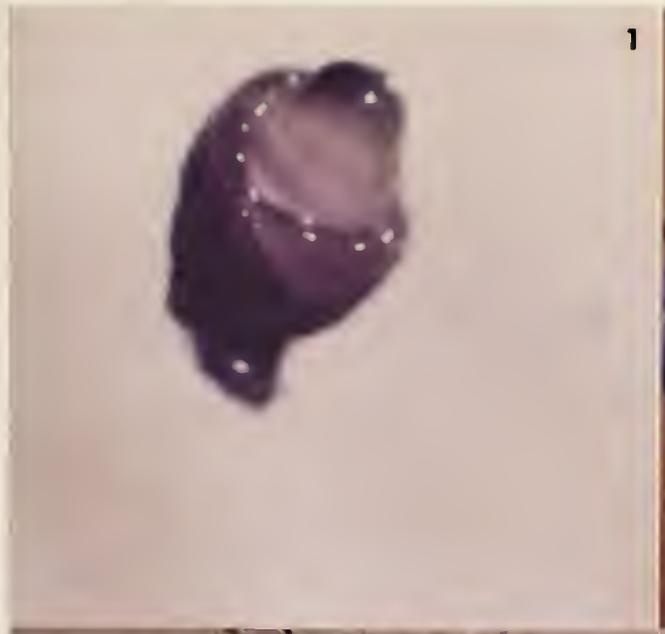
Figures 3-5. Method of colchicine application.

PLATE I

Figure 1. Growing point of Ae. comosa at first leaf stage.

Figure 2. Growing point of Ae. comosa at third to fourth leaf stage.

Figures 3-5. Method of colchicine application.


1


2


3


4


5

of paired chromosomes (bivalents and multivalents) and Z, the total number of cells analyzed. This formula is based on the fact that the shape of the curve formed by plotting the number of paired chromosomes against the number of chiasmata is a parabola. Thus by applying the data from a small sample of cells to the formula, one may obtain an estimate of the maximum amount of pairing from the extrapolate of the curve. In effect this would give the approximate pairing value that would be observed if a large sample had been analyzed.

(b) Mitosis

For karyotype studies of somatic cells root tips of the hybrids and parents were excised from the potted plants and treated in .002 M oxyquinoline at 15° C for 3 hrs. or in distilled water at 1° C for 12 - 15 hrs. Whenever possible parallel treatments were made of the parents and hybrids in order to obtain comparable results. The root tips were fixed in acetic alcohol (1:3) for 3 - 24 hrs., stained by the Feulgen method and mounted in a mixture of 1 part aceto-orcein : 1 part 45% acetic acid : 1 part glycerol.

Photographs of cells were taken at a magnification of 400X on 35 mm. Kodak High Contrast Copy film or Agfa Agepe film with a Zeiss Mikromat photomicroscope. A photograph of a slide micrometer scale was also taken at the same magnification to provide a scale for measuring absolute lengths. Enlargements were made from a negative of a metaphse plate and the micrometer scale on a single sheet of Kodak Resisto Rapid 8 x 10 in. paper by means of a double exposure. With these enlargements it was possible to measure the chromosomes with a high degree of accuracy against a division of the enlarged micrometer scale as a standard reference.

The lengths of straight chromosomes were determined directly in millimeters with a metric rule. For curved chromosomes a divider from a

standard drafting set was used. The points were set 2 mm. apart and the divider was "walked" along the main axis of the chromosome. The number of steps multiplied by 2 gave the metric length of the chromosome. In order to determine the absolute length in microns, the chromosome length in millimeters was then divided by the length of one micrometer scale division and the result was multiplied by 10 (1 division = 10 microns). This method gave a very low final error for a given mechanical error in measurement. For instance an inaccuracy of 1 mm. in measurement was shown to give an error of only .23 microns and a large inaccuracy of 5 mm., an error of only 1.2 microns. Since squashing frequently stretches the constrictions, these clear areas were not included in any of the measurements.

It was essential in this approach to chromosome analysis to use an objective producing the flattest field possible without loss of resolution, in order to avoid distortion and magnified errors in the final enlargement. A 100X, 1.25 n.a. oil immersion planachromat objective used in combination with a Leitz 5 cm. Focotar 1:45 corrected enlarger lens for the enlargements was found to be very satisfactory.

In Table 7 the relative lengths for the parental chromosomes was determined by $\frac{\text{chromosome length}}{\text{nuclear total}} \times 100$ where chromosome length refers to the length of the individual chromosome under consideration and nuclear total refers to the total length of all the chromosomes in the cell under consideration. A similar procedure was followed to obtain the relative lengths of the chromosomes in the hybrid but with a modification of the denominator. Here the total length of all the chromosomes in the hybrid genome under consideration was determined and multiplied by 2 in order to provide figures comparable to those of the respective chromosomes in the parents where 2 such chromosomes of each (I, II, III, etc.) are present as compared with one in the hybrid.

RESULTS AND DISCUSSION

A. HYBRIDIZATION

1. Crossability

The results of hybridization between the various Aegilops species and S. cereale and of treatments to produce amphidiploids from the hybrids are presented in Tables 2 and 3. The diploid Aegilops species showed in general a considerably lower crossability (rate of seed set) with Secale than did the polyploids. The kernels were shrivelled, and did not germinate under normal germinating conditions, so that the embryos always had to be cultured in vitro to obtain hybrid plants. In contrast, the seed set in crosses involving most of the tetraploid Aegilops species were much higher and the germinability (rate of germination under normal conditions) was also high. Crossability and germinability in crosses involving the hexaploids were uniformly high. This tendency, which was also noted by Oehler (1934) and Kihara (1937), is probably involved with the disturbing effect of the rye genes on the developmental pattern of the endosperm. From this viewpoint it would be expected that the disturbance, with polar nuclei containing 4 basic sets of Aegilops chromosomes, would be less than with polars with only two basic sets.

The unusually high crossability with rye of Ae. squarrosa and Ae. comosa on the one hand and the exceptionally low crossability of Ae. caudata and the group containing the S genome on the other indicate a considerable difference in the divergence of these species from rye. The fact that Ae. comosa had a relatively high seed set whereas the other two members of the M group, Ae. uniaristata and Ae. mutica have very low seed sets, indicates that perhaps this genome is diverging. The species containing the S genome appear thus to have developed almost complete barriers to crossability with rye, whereas Ae. squarrosa and the M group appear to be in the early stages of this process. The genome symbols are used after Kihara (1937).

TABLE 2

RESULTS OF CROSSING DIPLOID SPECIES OF AEGILOPS WITH SECALE CEREALE AND OF COLCHICINE TREATMENTS OF THE HYBRIDS

Diploid species	Genomes	No. florets pollinated	Seed set No. (%)	Number seeded	Number germinated	Number matured	Colchicine treated	C_1 seeds	C_2 seeds
Ae. caudata	C	152	0 (0.0)						
Ae. umbellulata	C^u	68	12 (17.6)	8	0				
Ae. squarrosa	D	271	129 (47.6)	43	2	1	1	1	45
Ae. comosa	M	115	35 (30.4)	8	1	1			
Ae. uniaristata	M^u	67	2 (3.0)	2	0				
Ae. mutica	M^t	36	0 (0.0)						
Ae. speltoides	S	488	16 (3.9)	0					
Ae. bicornis	S^b	353	25 (7.1)	2	2	0			
Ae. longissima	S^l	701	19 (2.7)	9	2	0			
Ae. sharonensis	S^l	256	2 (0.8)	0					

TABLE 3

RESULTS OF CROSSING TETRAPLOID AND HEXAPLOID AEGILOPS SPECIES WITH SECALE CEREALE AND OF COLCHICINE TREATMENTS OF THE HYBRIDS

Tetraploid species	Genomes	No. florets pollinated	Seed set No. (%)	Number seeded	Number germinated	Number matured	Colchicine treated	C_1 seeds	C_2 seeds
Ae. cylindrica	CD	56	5 (7.1)	2	2	2	1	2	55
Ae. ovata	C^uM^o	112	38 (33.9)	8	6	5	5	0	
Ae. triaristata	C^uM^t	147	57 (38.8)	30	23	12	6	0	
Ae. columnaris	C^uM^c	134	57 (42.5)	32	30	24	21	2	74
Ae. biuncialis	C^uM^b	117	44 (37.6)	21	18	14	13	1	7
Ae. variabilis	C^uS^v	281	6 (2.1)	4	2	1	2	1	48
Ae. triuncialis	C^uC	148	35 (23.6)	15	14	12	7	10	
Ae. crassa	DM^{cr}	73	19 (26.0)	5	0				
Ae. ventricosa	DM^v	210	37 (17.6)	13	0				
Hexaploid species									
Ae. triaristata	$C^uM^tM^t$	187	47 (19.8)	39	29	16	3	0	
Ae. crassa	DDM^{cr}	73	39 (53.4)	18	11	8	8	0	
Ae. juvenalis	C^uM^jD	88	42 (47.7)	15	4	3	6*	1	1

*cold-treated

2. Embryo cultures

Although abnormal seed development was a frequent occurrence in most of the crosses, it was more pronounced in some. For instance in crosses with Ae. ventricosa, all of the seeds were deficient to some degree in endosperm content. The majority contained a fluid endosperm and an immature and amorphous embryo. Tetraploid Ae. ovata and Ae. triaristata were also in this class. Hexaploid Ae. crassa however usually had a solid endosperm but it was frequently granular and segmented. Generally the crosses involving diploid Aegilops had a fluid endosperm in almost every instance, whereas those with the tetraploids had both solid and fluid endosperms and those with hexaploids had mainly solid endosperms.

The gradual transition from fluid to solid endosperm, paralleling the increase in ploidy, indicates this is one aspect of the disturbing effect of the Secale genome. In its general nature, this kind of endosperm failure is very similar to that reported by Rutihauser and La Cour (1956) in Paris x Trillium crosses and Beaudry (1951) in Elymus x Agropyron. Since there was strong evidence that the immediate causes in these instances were spontaneous breakage or loss of chromosomes and spindle abnormalities, it appears probable that they are the same in these Aegilops-Secale crosses. The gradation of effect and the resultant production of seeds varying in the severity of both endosperm and embryo abnormalities mentioned under Materials and Methods (Crosses and Embryo Cultures), moreover, indicates that endosperm failure is merely one aspect of a variable syndrome of effects upon the entire developing caryopsis.

In this project the limiting factor in the unsuccessful crosses, especially among the diploid Aegilops, appears to be a genetic imbalance within the embryo rather than an endosperm deficiency. This is indicated by the high frequency of grossly abnormal embryos which most probably would not

germinate on even the most refined media. The occasional appearance of a relatively normal embryo among these suggests that genetic heterogeneity of one or both parents may allow the production of occasional viable combinations of gametes.

3. Colchicine treatments

After several preliminary trials employing two of the more commonly used methods of colchicine application (Bell 1950, Sears 1951), the vacuum method suggested by Gentcheff (1955) was selected as the one most likely to yield results. Early experiments with a water soluble dye in place of colchicine determined the effectiveness of this method in bringing the colchicine into contact with the growing point. Growing points from the first to the fourth leaf stage were all deeply stained after being treated by the modified procedure described earlier.

All of the plants treated with colchicine by the vacuum method showed the usual signs of polyploidy, viz. swelling at the site of the growing point and subsequent enlargement of the leaves. However, considering the number of plants treated, the yield of successfully doubled plants leading to fertility was very low (Table 4). Many tillers showed signs of extreme polyploidy and these generally turned necrotic and died. Varying the concentration of colchicine or the duration of treatment did not increase the incidence of doubled sectors.

It should be pointed out that no conclusions can be made about the relative responses of the hybrids to colchicine from Tables 2, 3 and 4 due to the fact that cloning was carried out in those crosses where only one or two hybrids were obtained. For instance in the squarrosa - cereale hybrid, although only one hybrid was obtained, 8 clones were involved in the treatments.

TABLE 4

SUMMARY OF METHODS AND RESULTS OF INDUCING AMPHIDIPLOIDS

F_1 hybrid	Number treated	Colchicine conc. (%)	Period of Infiltration In vacuum	Period of Infiltration After vacuum	No. of polyploid seeds
Ae. squarrosa x *S. cereale*	10	0.05	5 min.	--	1
Ae. cylindrica x *S. cereale*	7	0.05	5 min.	--	3
Ae. columnaris x *S. cereale*	2	0.10	5-10 min.	10 min.	0
	4	0.05	5 "	-	0
	8	0.035	5-10 min.	-	2
	4	0.025	5 "	10 min.	0
Ae. variabilis x *S. cereale*	1	0.05	5 min.	-	1
	1	0.035	10 "	15 min.	0
Ae. biuncialis x *S. cereale*	7	0.05	5 min.	0-10 min.	0
	4	0.035	5 "	0-15 "	1
	3	0.025	10 "	5 "	0
Ae. juvenalis x *S. cereale*	3	Cold treated at 1°C for 72 hrs.			1

B. COMPARATIVE MORPHOLOGY AND CYTOTAXONOMY OF THE PARENTS, HYBRIDS AND AMPHIDIPLOID DERIVATIVES

I. Ae. comosa Sibth. et Sm. x S. cereale L. cv. Prolific

All of the seeds had either a degenerate or fluid endosperm and their embryos showed a wide range of abnormalities. However, one of the embryos appeared to be well-formed, having a regular scutellum and a relatively normal hypocotyl and epicotyl. All of the embryos were cultured but only the exceptional one germinated and grew to maturity.

(a) Plant morphology

Under greenhouse conditions Ae. comosa grew to a height of 27 cms. and the hybrid, 19 cms. The hybrid was weak and grew very slowly, requiring 152 days to head. Typical spikes of the parents and hybrid are shown in Plate II, Figs. 1 -3.

The glumes of Secale are keeled, rudimentary structures resembling lanceolate bracts, whereas those of Ae. comosa are non-keeled and well-developed, enclosing the spikelet completely. The glumes of the hybrid, although much narrower, nevertheless bear a much closer resemblance to those of Ae. comosa than to those of rye. In the lower spikelets of Ae. comosa the glumes are distally bifid, the two cleft portions tapering sharply to a beak-like tip and an apiculate shoulder of equal length. In the hybrid the glumes of the lower spikelet have a strong sub-median keel leading to the beak, which elongates to form an awnlet. The shoulder, although apiculate, is more reduced than that of Ae. comosa.

The glumes of Ae. comosa have denticulate nerves but no keel, whereas the glumes of Secale lack nerves but have a denticulate keel. The hybrid has both nerves and keel, each denticulate.

In the lower spikelets of Ae. comosa, the lemmas are bifid, but

PLATE II

1. Pistillate parent *Aegilops comosa* Sibth. et Sm.
2. Staminate parent *Secale cereale* L. c.v. Prolific.
3. F_1 hybrid of *Ae. comosa* x *S. cereale*.

1 2 3

in the hybrid they have the same appearance as the hybrid glumes, viz. awn-letted with apiculate shoulders. The lemmas of Secale are strongly keeled and taper to a shoulderless apex and a long awn. The lemmas of the hybrid are also strongly keeled and in this respect resemble the Secale parent.

In the apical spikelet of Ae. comosa, the glume of the primary floret is distally trifid, the cleft members tapering gradually into long flat awns, the lateral ones being equal in length and shorter than the median one. The glume of the secondary floret, on the other hand, is entire and tapers into a flat awn considerably longer than those of the primary floret. Characteristically the glume awns are erect and parallel with the main axis of the spike when green. However, at maturity they bend, the median awn of the primary floret at an angle of 90° and the awn of the secondary floret at an angle of 135°. The lemmas of both apical florets are trifid and resemble the primary glume on a much reduced scale.

The apical spikelet of the hybrid differs markedly from Ae. comosa in that the glumes of both primary and secondary florets are entire, like the secondary glume of Ae. comosa. Both glumes extend and taper gradually into long flat awns, and therefore appear much like the lemmas of the apical spikelet of rye. The lemmas each have an awn longer than the glume awn, with a minute apiculate shoulder on each side of it. In contrast to those of Ae. comosa, the awns of the hybrid do not bend at maturity but remain parallel to the main axis of the spike.

The palea of both Secale and Ae. comosa have two keels. The palea of the hybrid resembles that of Secale more closely in form and structure rather than Ae. comosa.

The hybrid bears a greater resemblance to the female parent Ae. comosa than to S. cereale in its gross appearance and in the details of spike morphology (Table 5). Fifteen of the 23 characteristics studied are similar

TABLE 5

MORPHOLOGICAL COMPARISONS OF *AE. COMOSA*, *S. CEREALE* AND THEIR F_1 HYBRID

Character	*Ae. comosa*	*S. cereale*	F_1 hybrid
Spike length	3.0 cms.	9.5 cms.	2.8 cms.
Spikelets	3-4	15-17	3-4
Florets per spikelet	3	2-3	3
Rudimentary spikelet	present	absent	present
Apical spikelet			
Glume nerves	6-7, denticulate	absent	6, denticulate
Glume type (primary)	complete, trifid	rudimentary, lanceolate	complete, lanceolate
(secondary)	complete, lanceolate	rudimentary, lanceolate	complete, lanceolate
Glume keel	absent	median	median
Glume width	wide	narrow	narrow
Glume shoulder (primary)	apiculate	wanting	apiculate
(secondary)	wanting	wanting	wanting
Glume beak (primary)	long, straight, denticulate	short, straight, denticulate	long, straight, denticulate
(secondary)	long, straight, denticulate	short, straight, denticulate	long, straight, denticulate
Lower spikelets			
Glume nerves	7-8, denticulate	absent	3-4, denticulate
Glume type	complete, bifid	rudimentary, lanceolate	complete, non-cleft
Glume keel	absent	median, strong	sub-median, strong
Glume width	wide	narrow, lanceolate	narrow
Glume shoulder	apiculate	wanting	apiculate
Glume beak	short, curved denticulate	short, straight, denticulate	short, curved, denticulate

to those of Ae. comosa, 7 to S. cereale and one is intermediate. Thus it appears that Ae. comosa has twice as many dominant genes as Prolific rye within the range of the characters in this study. However, this is probably an over-simplification of the true situation. The presence of quantitative characteristics as well as qualitiative ones reduces the certainty of an estimate of parental dominance in the hybrid. For instance the presence of a keel on the glume of rye and its absence in Aegilops is a qualitative characteristic. It is present in the hybrid and is considered to be conditioned by a single gene. On the other hand the shape of the glumes is a quantitative characteristic, the majority of characters involved being either comosa dominants or, alternatively, controlled by multiple-gene systems with the dosage in favour of the comosa parent. Other investigators of intergeneric hybrids (c.f. rev. lit.) found that the morphological picture ranged from dominance and intermediacy to transgression, with intermediacy being reported most frequently.

(b) Karyotype analysis

The measurements of chromosomes in Table 6 and the computations derived from them in Table 7 provide a basis for the identification of the parental chromosomes and, subsequently, the identification of the same chromosomes in the hybrid. The presence of characteristic satellites in the parents serve as markers for the identification of certain chromosomes, and the comparisons of total lengths, relative lengths and arm ratios are especially helpful in distinguishing the chromosomes both between and within the two genomes present in the hybrid.

Each determination is the average of 7 measurements obtained from a study of 7 cells. The primary and secondary constrictions were not included in the measurements, and the total chromosome lengths were obtained by adding the lengths of the long and short arms and the satellites.

TABLE 6

MITOTIC CHROMOSOME LENGTHS IN MICRONS OF *AE. COMOSA*, *S. CEREALE* AND THEIR F_1 HYBRID

Chrom. no.	Source	*Ae. comosa* genome				*S. cereale* genome			
		Long arms	Short arms	Satellites	Total lengths	Long arms	Short arms	Satellites	Total lengths
I	Parent	4.4 ± .1	4.0 ± .1		8.4 ± .1	5.9 ± .2	5.0 ± .1		10.9 ± .3
	F_1	3.2 ± .1	2.9 ± .1		6.0 ± .2	4.7 ± .2	4.4 ± .1		9.0 ± .2
II	Parent	3.7 ± .1	2.6 ± .1	2.0 ± .0	8.3 ± .1	5.9 ± .1	4.5 ± .2		10.2 ± .3
	F_1	2.6 ± .2	1.9 ± .1	1.3 ± .1	5.8 ± .3	5.2 ± .4	3.5 ± .2		8.7 ± .4
III	Parent	5.7 ± .1	2.4 ± .1		8.0 ± .2	6.8 ± .2	2.8 ± .1	0.6 ± .0	10.2 ± .2
	F_1	3.9 ± .1	1.7 ± .1		5.6 ± .1	4.8 ± .1	3.6 ± .2		8.4 ± .4
IV	Parent	5.3 ± .1	2.3 ± .1		7.6 ± .1	6.0 ± .2	4.0 ± .1		10.0 ± .2
	F_1	3.7 ± .2	1.7 ± .1		5.5 ± .1	4.9 ± .3	3.3 ± .2		8.2 ± .4
V	Parent	4.1 ± .1	3.3 ± .1		7.4 ± .1	5.3 ± .1	4.4 ± .1		9.7 ± .3
	F_1	2.8 ± .1	2.4 ± .2		5.2 ± .3	5.0 ± .3	2.8 ± .2		7.9 ± .0
VI	Parent	4.0 ± .1	2.0 ± .1	0.7 ± .1	6.6 ± .0	6.2 ± .2	2.6 ± .1	0.5 ± .0	9.4 ± .3
	F_1	2.9 ± .3	1.4 ± .1	0.6 ± .0	4.9 ± .3	5.0 ± .2	2.7 ± .3		7.7 ± .3
VII	Parent	3.8 ± .1	2.8 ± .0		6.6 ± .1	5.4 ± .2	2.2 ± .1	1.1 ± .1	8.7 ± .3
	F_1	2.8 ± .1	2.0 ± .1		4.8 ± .2	4.5 ± .2	2.9 ± .2		7.4 ± .4

Numbers I to VII were assigned to the chromosomes in both parental species according to their descending order of lengths; in the hybrid, where two non-overlapping ranges including 7 chromosomes each were found, two sets of numbers I to VII were used. The results of calculations of relative lengths, arm ratios and coefficients of variability from the data and the methods of calculation are shown in Table 7. The satellites were not included in the calculation of arm ratios. The block idiogram in Plate IV, prepared from the data in Table 6, is arranged in a form that permits a ready comparison of the parental and hybrid chromosomes.

By arranging the chromosomes in descending order of size and converting the absolute measurements to relative lengths a convenient standard approach is possible. By means of this conversion, the experimental variations in chromosome length from mucleus to nucleus are eliminated. For example, the total length of chromosome I in Ae. comosa is 8.40 microns, but due to prolonged cold treatment, the same chromosome in the hybrid is 6.02 microns. However, the relative lengths of these chromosomes is 7.9 and 8.0 percent respectively. Chromosome II also has a relative length of 7.9 percent but it can be distinguised from I by the difference in arm ratios. The relative lengths, therefore, are of value in distinguishing one chromosome from another within the same genome and for matching corresponding chromosomes from the parents and hybrid.

The coefficients of variability shown in Table 7 give an indication of the variability in total length of a given chromosome from nucleus to nucleus. This is actually the sample standard deviation expressed as a fraction of the sample mean and converted to percentage. Since each biological entity has a characteristic coefficient of variability, it is necessary to have experience with similar data to determine whether or not a particular coefficient is unusually large or small. If experience determined

TABLE 7

RELATIVE LENGTHS, ARM RATIOS AND COEFFICIENTS OF VARIABLITY IN AE. COMOSA, S. CEREALE AND HYBRID CHROMOSOMES

		Ae. comosa genome			*S. cereale* genome		
Chromosome number	Source	Relative* lengths	Arm ratios	Coefficients of variablity	Relative* lengths	Arm ratios	Coefficients** of variablity
I	Parent	7.9	1.1	4.7	7.9	1.2	10.6
	F_1	8.0	1.1	9.1	7.9	1.1	4.6
II	Parent	7.9	1.4	4.5	7.5	1.3	9.1
	F_1	7.7	1.4	13.9	7.6	1.5	12.3
III	Parent	7.6	2.4	6.8	7.4	2.5#	7.9
	F_1	7.5	2.2	4.0	7.4	1.4#	12.2
IV	Parent	7.2	2.3	7.0	7.2	1.5	8.9
	F_1	7.2	2.2	4.3	7.1	1.5	11.9
V	Parent	7.0	1.2	7.0	7.0	1.2	10.8
	F_1	6.9	1.2	15.6	6.9	1.8	13.1
VI	Parent	6.2	2.0	6.5	6.8	2.4#	13.7
	F_1	6.5	2.0	13.8	6.7	2.0#	11.8
VII	Parent	6.3	1.4	5.2	6.3	2.5#	10.8
	F_1	6.3	1.4	12.8	6.5	1.6#	13.9

*F_1 relative length: $\frac{\text{Chromosome length}}{2(F_1 \text{ genome total})} \times 100$

Parent relative length: $\frac{\text{Chromosome length}}{\text{nuclear total}} \times 100$

**Coefficient of variablity: $S\left(\frac{100}{\bar{X}}\right)$ where S = standard deviation, $\bar{X}$ = sample mean

#Deviation from parental ratio due to incorporation of satellite in hybrid

the range of variability to be 5 to 15 percent, then values outside of this interval would cause the investigator to wonder if an error had been made in calculation, or if some unusual circumstances threw doubt on the validity of the experiment. In view of the fact that no other karyotype analyses of this nature containing coefficients of variability are available, there is no means of evaluating the variability encountered in this experiment. However the data indicate that the coefficients, on the average, are somewhat higher in the hybrid than in the parents, suggesting that differential contraction may depend on the degree of adjustment of some chromosomes to the new genomic combination. This is an aspect that requires further study

The karyotype of Ae. comosa has been studied and reported by three investigators and each has found new characteristics. In Sorokina's (1928) study no satellites are described. However, in Senjaninova-Korczagina's report (1932) a pair with large satellites are described. In Chennaveeraiah's (1960) study, a 'chromosome II' with a secondary constriction giving an arm ratio of 1:1:1.5, presumably the same pair as that of Senjaninova-Korczagina's, is described. A small pair of satellites on chromosome VI is also reported by Chennaveeraiah.

In the present study, the locations of the satellites on chromosomes II and VI are in agreement with Chennaveeraiah's description, except that the large satellite differs somewhat. In the preparations in which cold treatment was used for shortening the chromosomes a constriction appeared across the large satellite on chromosome II (Plate III, figs. 1, 2) and when it became separated from the main body of the chromosome, it assumed the appearance of a B chromosome with a median centromere. There is also general agreement between the arm ratios of chromosomes II, V, VI, and VII, and if chromosomes I and III were interchanged, these would also agree. This leaves chromosome IV as the only source of difference between the two

idiograms, Chennaveeraiah's chromosome IV being median and that in this study, sub-median. Since the arm ratios were not presented in Chennaveeraiah's report, the idiogram presented therein was measured to derive arm ratios for comparison. It is indeed rather surprising that greater differences were not found between these two studies in view of the fact that one was based on measurements from a single cell, and the other from the mean of seven cells.

It is apparent that the chromosomes of Ae. comosa are comparatively uniform in length but are morphologically distinct. Although the mean difference between the longest and the shortest is only 1.8 microns, all seven chromosomes of the haploid set are nevertheless readily identified by their characteristic arm ratios, relative lengths and the presence or absence of satellites.

Considerable disagreement between various workers concerning the karyotype of S. cereale renders a comparison for the purpose of determining the validity of the karyotype presented here difficult. However, it does agree closely with that described by Battacharyya and Jenkins (1960). In their study, based on measurements from 10 cells, the chromosomes of S. cereale show the same uniformity in length and morphological distinctness. The mean difference between the longest and shortest chromosome is again slight, and all of the chromosomes except IV and V, which are closely similar, may be readily identified by their relative lengths, arm ratios and the presence or absence of satellites. The largest satellite, on chromosome VII, and the smallest, on chromosome VI, are readily seen in most cells but that on chromosome III does not appear consistently. Occasionally single secondary constrictions appear in the long arms of chromosomes III and V but not with sufficient frequency to be considered reliable markers. In view of these close agreements, therefore, it may be concluded that the karyotype presented here is a rather accurate one.

PLATE III

Photomicrographs and photodrawings of somatic metaphases from parents and F_1 hybrid of *Ae. comosa* x *S. cereale*

Figures 1, 2. Pistillate parent *Ae. comosa*.

Figures 3, 4. Staminate parent *S. cereale*.

Figures 5, 6. F_1 hybrid *Ae. comosa* x *S. cereale*.

Arrows indicate chromosomes of *S. cereale*.

1
2
3
4
5
6

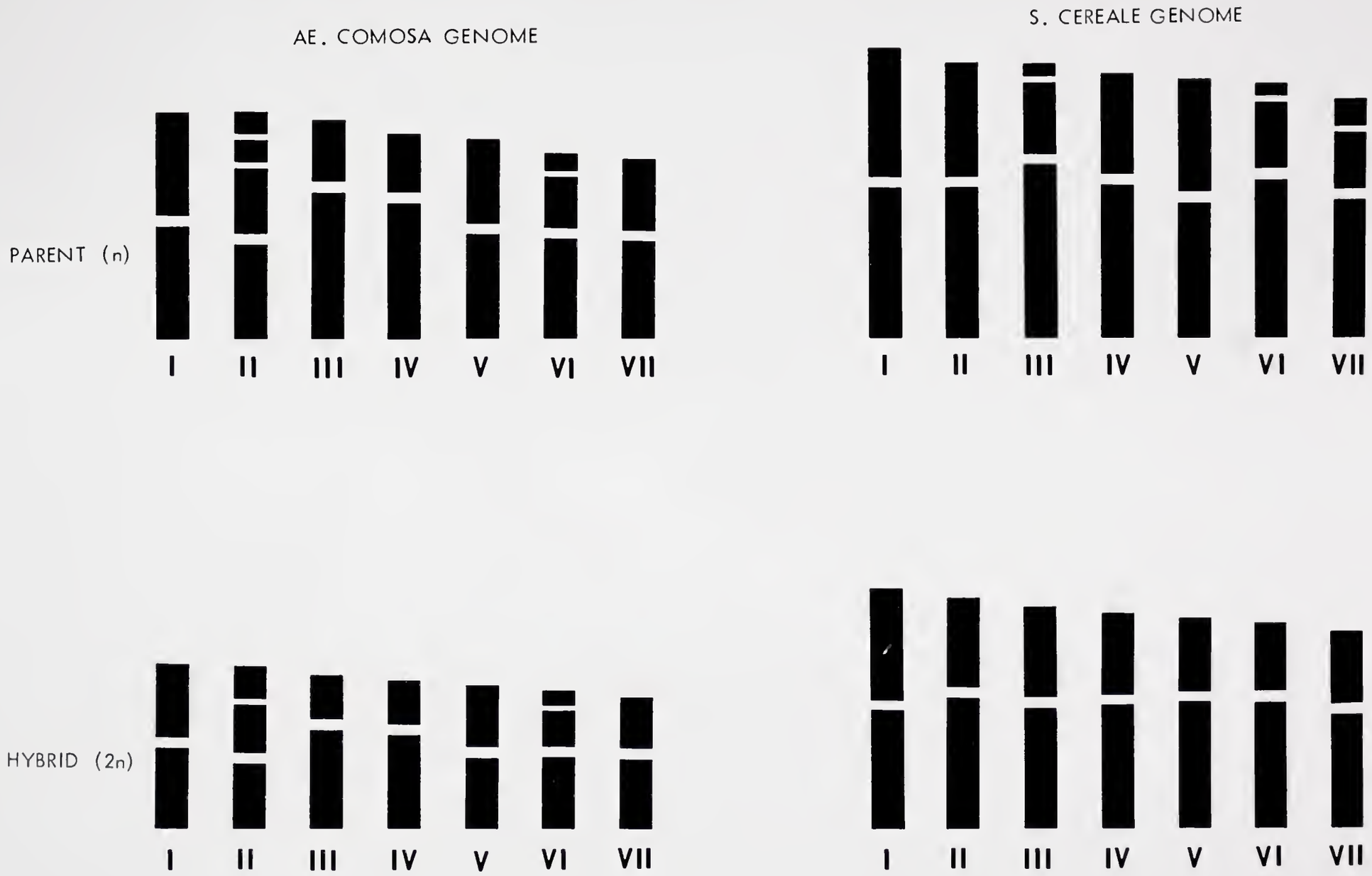
The smaller group of chromosomes in the hybrid correspond very closely with those of Ae. comosa. The satellites on chromoses II and III correspond (see Plate IV), and the arm ratios bear an extremely close agreement throughout (Table 7). Therefore,considering the measurements together with their standard errors, there is no overlapping of the longest comosa chromosome with the shortest rye chromosome. The largest comosa chromosome (I) is still significantly shorter than the smallest rye chromosome (VII), there being no overlap when the lengths and their standard errors are compared either in the parents or in the hybrid. In the hybrid these size differences are not only maintained but are also accentuated by a differential response to the cold treatment used in shortening the chromosomes, the comosa chromosomes being shortened more than those from Secale. It may therefore be concluded that the smaller group of 7 chromosomes in the hybrid are from Ae. comosa and that the individual comosa chromosomes may be accurately distinguished from each other by measurements of comparative length. This observation, together with the observation that there is a very close correlation in relative lengths between the chromosomes of S. cereale and the larger group of chromosomes in the hybrid renders it certain that this group is entirely of cereale origin. On the basis of this evidence it is possible to identify the parental chromosomes in the F_1 hybrid.

In the hybrid, two morphological changes in the chromosomes are of particular interest. The constriction which is present on the satellite of chromosome II in Ae. comosa is not evident in the hybrid. Whether this is a physiological effect of the combination of two diverse genomes or whether the treatment used in this study was not suitable for the expression of this constriction is not known. The other feature of interest is the failure of the rye satellites to appear in the hybrid. Although the two satellites of Ae. comosa appear consistently, those of rye do not, but appear to be

PLATE IV

Block idiogram of parents and F_1 hybrid of *Ae. comosa* and *S. cereale*.

BLOCK IDIOGRAM OF PARENTS AND F_1 HYBRID OF AE. COMOSA AND S. CEREALE



incorporated into the short arm, thereby causing a change in the arm ratios for these chromosomes. This phenomenon will be discussed in some detail in the section on the hybrid of Ae. squarrosa and S. cereale.

(c) Meiotic analysis

Having established the fact that the chromosomes of Secale cereale are longer than those of Ae. comosa both in the parental and hybrid somatic cells, the question arises whether the same situation exists in the meiotic cells. During meiosis there are no recognizable markers on the chromosomes with which to distinguish the cereale genome from the comosa genome. The only apparent differences are those of chromosome size, the chromosomes being readily classified into two groups, 7 long and 7 short. It is at this point that the assumption must be made that the size differential between the parental chromosomes in the hybrid is maintained during meiosis in approximately the same proportions as during mitosis. Thus the 7 long chromosomes represent the cereale genome and the 7 short ones represent the comosa genome. It is highly improbable that a chromosome of the comosa genome, for example, would contract differentially and appear like a cereale chromosome. In support of this assumption some of the previous work involving hybrids from species with chromosomes of different size may be recalled, especially that of Brown (1958), Wagenaar (1959), Hakansson (1943) and Stebbins and Pun (1953) all of whom agreed that the somatic size-relationships of the parental chromosomes were maintained during meiosis.

The classification of metaphase chromosome associations during meiosis as heteromorphic or homomorphic bivalents yields some interesting results regarding intergeneric pairing (Table 8). The majority of the cells (54 percent) show no apparent chromosomal associations, the univalents appearing to lie at random throughout the cytoplasm. In 39 percent of the cells, associations between Secale and Aegilops chromosomes appear, and only

rarely does autosyndesis occur. Trivalents, although present, are equally rare. Side-by-side associations, as analyzed by Person (1955) and Riley and Chapman (1957), are not encountered. Typical meiotic cells are shown in Plate V.

An estimation of the number of chromosomes potentially capable of pairing by Gaul and Münzner's formula is 6.6. In other words each cell contains on the average 6.6 chromosomes that may form bivalents or trivalents. Assuming that bivalents only occur, there is a potential of 3.3 bivalents per cell on the average. Actually the average bivalent association as seen in the data is only 0.48 per cell. It is this discrepancy with which we are mainly concerned and which is discussed at length in a succeeding section.

TABLE 8

FREQUENCIES AND TYPES OF CHROMOSOMAL ASSOCIATIONS IN AE. COMOSA X S. CEREALE

No. of cells	Univalents		Bivalents			Trivalents	
	A*	S*	AA	SS	AS	AAS	ASS
52	7	7					
30	6	6			1		
4	7	5		1			
4	5	7	1				
3	6	5					1
1	5	6				1	
1	5	5			2		
1	4	4	1	1	1		
Totals 96	624	622	5	5	33	1	3
Average per cell	6.5	6.4	.05	.05	.34	.01	.03

* A = Aegilops
  S = Secale

PLATE V

Meiosis in the F_1 hybrid of *Ae. comosa* x *S. cereale*.

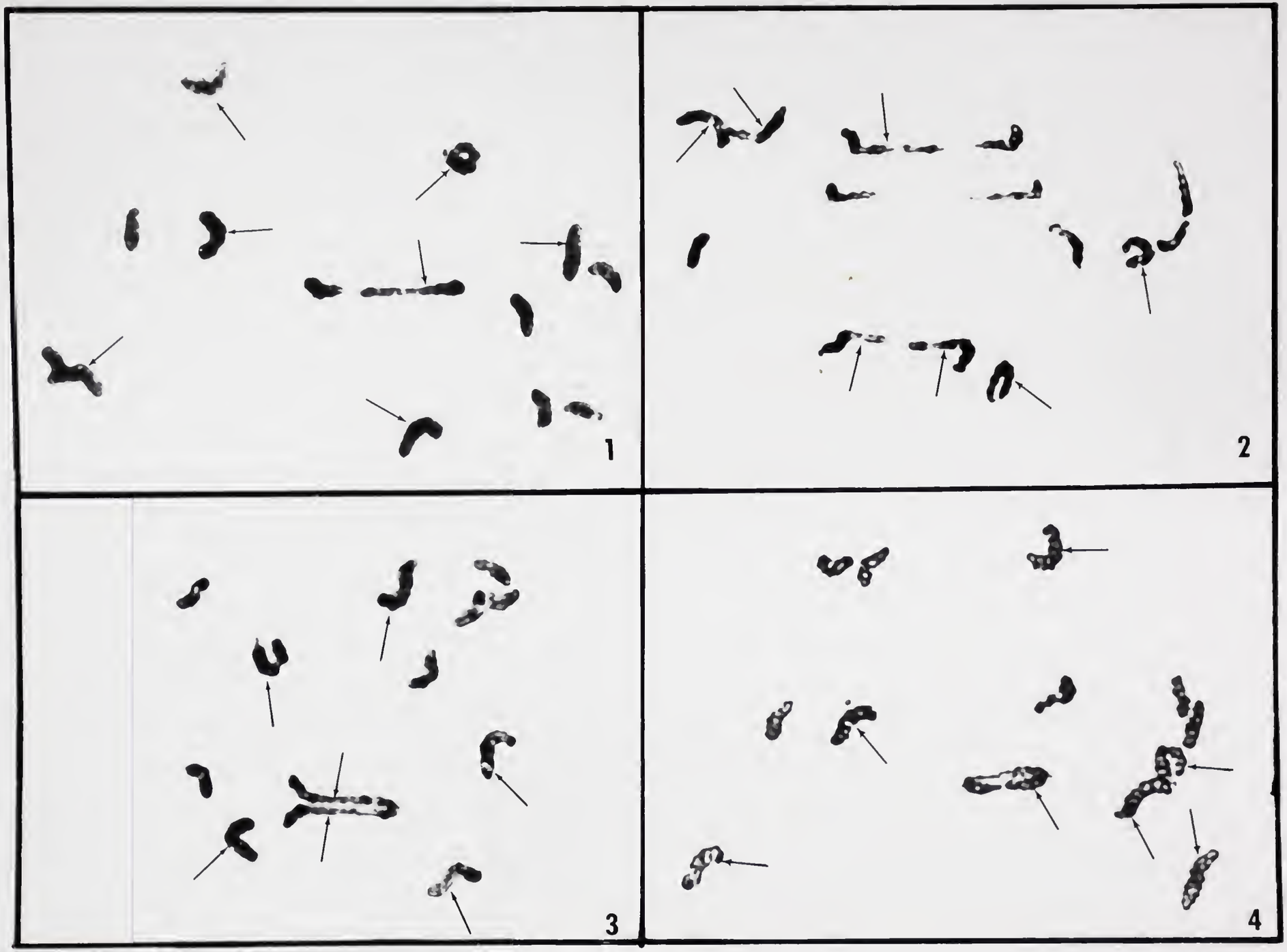
Figure 1. An *Aegilops-Secale* rod bivalent.

Figure 2. An *Aegilops-Secale* bivalent, an *Aegilops* bivalent and a *Secale* bivalent

Figure 3. A trivalent composed of 2 *Secale* and 1 *Aegilops* chromosomes.

Figure 4. An *Aegilops-Secale* ring bivalent.

Arrows indicate chromosomes of *S. cereale*.


1
2
3
4

II. Ae. squarrosa L. var. typica x S. cereale L. c v. Prolific

(a) Parents and F_1 hybrid

1. Plant morphology

The description of Ae. squarrosa L. given by Nevski (1934) and translated from the Russian by Turin (1940), fits the species Ae. squarrosa var. typica used in this experiment. It is an annual with many culms, geniculate in the lower part, and 20 to 40 cm. tall. The leaves are glabrous or sparsely hirsute. The spikes are linear and cylindrical and disarticulate easily at maturity. Spikes of the parents, hybrid and the C_1 amphidiploid are shown in Plate VI, figs. 1-4. A comparison of some of the characteristics is presented in Table 9.

This hybrid differs from the previous one in that it resembles the rye parent more than the squarrosa parent in its gross morphology. Of the 12 characteristics studied, it resembles S. cereale in 7, Ae. squarrosa in one, is intermediate in 3 and shows transgression in spike length. The first generation amphidiploid shows little or no difference from the F_1 hybrid except in a slight increase in spike length and slightly more pronounced spike characteristics.

The comparative morphology of the hybrid is of considerable interest because it gives an indication of some of the genetic relationships between two haploid sets of chromosomes from different genera and allows a comparison between the squarrosa and comosa sets against a common cereale background.

The spike of Ae. squarrosa has an extremely fragile rachis with disarticulation occurring at each node. Neither Secale nor the hybrid are disarticulate however. On the other hand, the spike of the comosa-cereale hybrid is disarticulate at the base like the comosa parent. The transgression in spike length of the squarrosa - cereale hybrid was not found in the comosa-cereale hybrid, which rather approached the comosa parent in length.

PLATE VI

1. Pistillate parent *Aegilops squarrosa* L. var. typica.
2. Staminate parent *Secale cereale* L. c.v. Prolific.
3. F_1 hybrid of *Ae. squarrosa* x *S. cereale* (2n = 14).
4. C_1 amphidiploid of *Ae. squarrosa* x *S. cereale* (2n = 28).

1 2 3 4

TABLE 9

COMPARATIVE MORPHOLOGY OF *AE. SQUARROSA*, *S. CEREALE*, F_1 HYBRID AND C_1 AMPHIDIPLOID

Character	*Ae. squarrosa*	*S. cereale*	F_1 hybrid	C_1 amphidiploid
Plant height	20-40 cm.	90-132 cm.	105 cm.	112 cm.
Spike length	8-11 cm.	9-11 cm.	16-18 cm.	16-18 cm.
No. of spikelets	7-15	28-32	28-32	21-25
Florets per spikelet	4-5	2-3	3	3
Awn length	0.5-2.5 cm.	0.5-4.5 cm.	1.0-5.0 cm.	1.0-5.0 cm.
Glume shape	rectangular	lanceolate	elliptical	elliptical
" nerves	7-10, denticulate	absent	4, denticulate	6-7 denticulate
" keel	absent	median, denticulate	sub-median, denticulate	sub-median, denticulate
" width	wide	narrow	medium	medium
" shoulder	obtuse-truncate	wanting	sloping	sloping
" beak	absent	short, straight, denticulate	short, straight, denticulate	short, straight, denticulate
Hairy neck	absent	present	present	present

The glumes of Ae. squarrosa are broad, truncate, nerved, lack a keel and completely cover the spikelet on one side. As indicated earlier, the rye glumes are rudimentary, bract-like structures and are keeled, nerveless and lanceolate in form. The glumes of the hybrid are similar in form to those of rye but are somewhat larger and broader. Although they resemble rye in general outline, they contain the keel of one parent and the nerves of the other. A similar situation with respect to the keel and nerves occurs in the comosa-cereale hybrid and parents.

The lemmas of the lower spikelets of Ae. squarrosa are broad and have a rounded shoulder which terminates in a short, recurved, thick awn. The upper lemmas have a nearly apiculate shoulder at the base of an awn which is considerably longer, thick, recurved and denticulate along both edges. The lemmas of rye are slender and taper gradually to form a long thin awn which is straight but also denticulate along both edges. The lemmas of the hybrid closely resemble those of rye with the exception of a minute, apiculate shoulder at the base of the awn. A similar shoulder appears on both sides of the awn at the base in the comosa-cereale hybrid.

2. Karyotype analysis

The somatic chromosomes of Ae. squarrosa in this study correspond generally with the description given by previous authors as reviewed by Chennaveeraiah (1960). The somatic chromosomes of Ae. squarrosa, S. cereale and the hybrid are shown in Plate VII, figs. 1 to 3. The karyotype of Ae. squarrosa contains 14 chromosomes, one pair of which contains satellites. As described earlier, S. cereale has 3 pairs of satellites. In the hybrid, the karyotype predominantly has only one satellite, that on the third squarrosa chromosome, although one rare cell showed 4 satellites, 3 on cereale chromosomes and one on a squarrosa chromosome (fig. 3). Table 10 shows the arm lengths, total lengths and arm ratios of parental and hybrid chromosomes

PLATE VII

Figure 1. Metaphase chromosomes from a somatic cell of Ae. squarrosa.

Figure 2. Metaphase chromosomes from a somatic cell of S. cereale.

Figure 3. Metaphase chromosomes from a somatic cell of Ae. squarrosa x S. cereale.

Figures 4 to 9. Meiosis in the F_1 hybrid of Ae. squarrosa x S. cereale.

Arrows indicate the chromosomes of S. cereale.
"S" indicates satellites.

1
S
S
2
S
S
S
S
S
3
S
S
S
4
5
6
7
8
9

each based on the measurements of 3 cells. As in the previous experiment, the satellites are shown separately and are not included with the short arm in the calculation of arm ratios.

The smaller group of chromosomes in the hybrid closely resemble those of Ae. squarrosa. The satellites on chromosome III correspond and the arm ratios are comparable throughout. It may be concluded therefore that the smaller group of 7 chromosomes in the hybrid are from Ae. squarrosa and that the individual squarrosa chromosomes may be accurately distinguished from each other by comparison of total lengths and arm ratios. The larger chromosomes compare favorably with the cereale haploid set of chromosomes in total lengths and the arm ratios also correspond in the non-satellited chromosomes. In the satellited chromosomes, however, the same situation of satellite dominance* appears to exist as was recorded for the previous hybrid. The satellites on chromosomes III, VI, and VII are not visible, and appear to be incorporated into the short arms of their respective chromosomes. This results in a change in the arm ratios for these chromosomes as noted in Table 10.

The failure of the cereale satellites to appear in this hybrid indicates that the phenomenon is not an isolated one peculiar to the previous hybrid alone. An examination of the first generation amphidiploid and the 8 progeny of the second generation reveals that the same suppression of rye satellites occurs. This characteristic is also reported by Evans and Jenkins (1960) in single rye chromosome additions to wheat. In the line containing 42 wheat chromosomes plus chromosome VII, the nucleolar

*The nuclear situation in a hybrid with 2 or more alien genomes in which satellited chromosomes from one genome dominate in the role of nucleolar organizers and those of the others fail to show their satellites.

TABLE 10

MITOTIC CHROMOSOME LENGTHS IN MICRONS IN *AE. SQUARROSA*, *S. CEREALE* AND THEIR F_1 HYBRID

		Ae. squarrosa genome					*S. cereale* genome				
Chrom. no.	Source	Long arms	Short arms	Satellites	Total lengths	Arm ratios	Long arms	Short arms	Satellites	Total lengths	Arm ratios
I	Parent	4.31	3.01		7.32	1.43	5.92	5.00		10.93	1.18
	F_1	4.30	3.14		7.44	1.37	6.05	5.58		11.63	1.08
II	Parent	3.52	3.33		6.70	1.06	5.90	4.48		10.38	1.32
	F_1	3.72	3.49		7.21	1.07	6.16	4.65		10.87	1.32
III	Parent	4.26	1.76	0.56	6.56	2.42	6.80	2.78	0.60	10.17	2.45
	F_1	4.53	1.98	0.60	7.11	2.29	6.74	3.72	-	10.46	1.81 *
IV	Parent	3.75	2.73		6.48	1.37	6.03	3.95		9.98	1.53
	F_1	3.85	2.79		6.74	1.42	6.51	3.72		10.23	1.75
V	Parent	3.66	2.32		5.97	1.58	5.29	4.37		9.66	1.21
	F_1	4.19	2.33		6.52	1.80	5.47	4.65		10.12	1.18
VI	Parent	3.43	1.95		5.37	1.76	6.23	2.61	0.50	9.39	2.39
	F_1	3.84	2.33		6.17	1.65	6.16	3.49	-	9.65	1.77 *
VII	Parent	2.74	2.64		5.38	1.04	5.37	2.19	1.10	8.66	2.45
	F_1	3.02	2.79		5.81	1.08	5.23	4.30	-	9.53	1.22 *

* Deviation in arm ratio due to incorporation of satellite into short arm

organizer of rye, either in the monosomic or disomic condition, the rye satellite was seldom seen. Only occasionally was a slight demarcation noticed where the constriction usually appears. The significance of this is not known although Evans and Jenkins believe that the function of the chromosome as a nucleolar organizer is not required when in combination with the wheat complement. Consequently, this loss of function results in a visible change in morphology.

This characteristic of satellite suppression has been reported earlier. Sveshnikova (1928) according to Darlington (1937) reported that the 28-chromosome race of Vicia cracca has the chromosome complement of the 14-chromosome race represented twice except that one pair have lost their satellites. This appears to be another instance of satellite dominance but in an autopolyploid. Darlington also cites McLintock's (1934) investigation of the nucleolar organizer in Zea wherein she suggests that there is competition for the available materials between two organizers and the more active one will dominate. Cited also is Navashin's (1934) work in Crepis hybrids which is analogous to the present study. Here a strong organizer from one parent seems to supplant a weaker one from the other. Since the nucleolus stretches the constriction, the weaker nucleolar chromosome fails to develop its characteristic long constriction in the hybrid. Similar competition is found in Drosophila in the attached XY + Y males where, according to Kaufman (1934), the attached arm of the Y does not develop its usual-sized nucleolus owing to the proximity of the organizer in the X chromosome.

It is interesting to note the dominance relationships of these satellites and to compare them with the satellites in common wheat. Sarkar and Stebbins (1956) proposed that Ae. speltoides is the donor of the B genome in wheat and Riley et al (1958) provided cytological evidence in support of this. The latter authors pointed out that 2 pairs of satellites in

Ae. speltoides resemble those in the tetraploid and hexaploid wheats, but that the satellites of Ae. squarrosa, which is accepted as the donor of the D genome, do not appear. Some authors Bhatia (1938), Pathak (1939) and Camara (1944) reported 3 pairs of satellites, the third pair presumably being from the A or D genome. However, Levitsky et al (1939), Tjio and Levan (1950) and Riley et al (1958) reported only 2 pairs. The total number of satellited chromosomes from the proposed parents of common wheat, T. aegilopoides (A), Ae. squarrosa (D), and Ae. speltoides (B), is 4 pairs. As 2 pairs only are seen, it appears that two others are suppressed. This relationship of satellite dominance is open to study between the A and B genomes and the A and D genomes since the amphiploids between them are available (Sears 1941).

3. Meiotic analysis

Meiosis in the parental species as studied in approximately 100 cells was normal. The meiotic preparations of the hybrid shown in Plate VII, figs. 4-9 revealed that the size-differences of the parental chromosomes were maintained in the hybrid during metaphase of meiosis. This asymmetry again provided a means of determining the nature of chromosomal pairing in the hybrid. Heteromorphic bivalents (Figs. 4, 5, 7, 8), trivalents (Figs. 6, 7), multiple associations (Fig. 9) and homomorphic pairs (Figs. 6, 8) could readily be scored for the squarrosa and cereale chromosomes present in each. The results of an analysis of 123 cells are presented in Table 11. Thirty-five percent of the cells had only univalents distributed at random. Twenty-four percent of the cells had one open bivalent formed by an Aegilops-Secale association and 13 percent had 2 similar bivalents each. Seventy percent of the total number of bivalents recorded were of intergeneric origin. According to Gaul and Munzner's formula, the number of chromosomes potentially capable of pairing in the hybrid is 12.6, whereas the average bivalent frequency per cell observed was only 1.12.

TABLE 11

FREQUENCIES AND TYPES OF CHROMOSOMAL ASSOCIATIONS IN AE. SQUARROSA X S. CEREALE F_1

No. of cells	Univalents		Bivalents			Trivalents			Quadrivalents
	A	S	AA	SS	AS	AAA	AAS	ASS	AASS
43	7	7							
5	5	7	1						
7	7	5		1					
24	6	6			1				
6	4	6	1		1				
8	6	4		1	1				
5	5	3		1	2				
1	3	5	1		2				
13	5	5			2				
1	6	5						1	
1	3	4	1	1				1	
5	3	6			1	1			
2	5	6					1		
2	5	5							1
Total 123	730	728	14	20	81	5	2	2	2
Av. per cell	5.9	5.9	.1	.2	.7	.04	.02	.02	.02

A = Aegilops
S = Secale

The evidence of intergeneric pairing of chromosomes in this hybrid strongly supports the conclusion reached earlier that such pairing occurred in the comosa-cereale hybrid. The heteromorphic nature of the majority of the bivalents was unmistakable. There appears to be considerably more pairing in the squarrosa-cereale hybrid than in the comosa-cereale hybrid, there being approximately 50 percent more bivalents per cell of both heteromorphic and homomorphic types in the former. This increased pairing frequency is reflected in the estimated pairing potential according to Gaul and Münzner's method (12.6 for the squarrosa-cereale hybrid and only 6.6 for the comosa-cereale hybrid). That cytological relationship between Ae. squarrosa and Ae. comosa is very close is indicated by the hybrid between them, which shows six bivalents. One might, therefore, expect somewhat similar results between each of their hybrids with rye. The fact that this is not obtained is possibly due either to segmental differences in their chromosomes or differences in genes controlling pairing and chiasma formation.

The number of chromosomes potentially capable of pairing, 12.6, is a very high estimate in view of the fact that there are only 14 chromosomes in the hybrid. This may lead to the misinterpretation that 6 chromosomes of squarrosa are capable of pairing with 6 of cereale. Actually, the occurrence of autosyndesis reduces these figures considerably, since only 62.6 percent of the chromosomes involved in general pairing formed intergeneric associations. Therefore a similar percentage of the estimated 12.6 may be expected to be potentially capable of forming intergeneric pairs, i.e. 7.9 chromosomes. In other words, approximately 4 chromosomes of Aegilops squarrosa are capable of pairing with 4 of Secale cereale. However the observed average bivalent frequency per cell was only 1.12. The reason for this reduction in the observed pairing frequency will be discussed at length in a following section.

(b) Amphidiploids

1. Plant morphology

Morphological characters of the first generation amphidiploid (C_1) are compared with those of the parental species and F_1 hybrid in Table 9. In plant height, there is a slight increase over that of the hybrid. The morphological characteristics of the spikes are a little more pronounced (Plate VI, figs. 3, 4). The spikes are free-threshing like the rye parent and the self-fertility is very low (.21%). The predominance of rye-like characteristics is also seen in the C_2 generation as well as in the F_1 hybrid and C_1 generation (Plate VIII, figs. 1-3).

Ten of the seeds from the C_1 plant were grown in the C_2 generation. One failed to germinate and another was an outcross to common wheat, leaving eight C_2 plants. In Table 12 the chromosome numbers, some of the morphological characteristics and the self-fertility of these plants are presented.

TABLE 12

SOME CHARACTERISTICS OF AE. SQUARROSA X S. CEREALE C_2 AMPHIDIPLOIDS

Plant no.	Chrom. no.	Height (cm.)	No. of culms	Av. no. spikelets*	Av. spike length*	Spike density	Seed set (%)
1	28	113	39	19.9	15.9	1.3	9 (0.68)
2	28	116	45	18.3	17.4	1.4	17 (1.03)
3	27[1]	98	28	22.1	17.0	1.4	44 (7.10)
4	27[2]	101	39	15.1	15.7	1.4	1 (0.08)
5	28	108	28	22.3	17.3	1.5	0 (0.00)
6	28	114	36	17.5	17.7	1.3	6 (0.40)
7	28	119	25	21.3	18.6	1.3	4 (0.40)
8	28	112	29	21.0	17.7	1.4	13 (1.00)

1 14 squarrosa and 13 cereale chromosomes

2 13 squarrosa and 14 cereale chromosomes

* values derived from a study of 10 spikes

PLATE VIII

Figure 1. F_1 hybrid of Ae. squarrosa x S. cereale.

Figure 2. C_1 amphidiploid.

Figure 3. C_2 amphidiploids.

No. 1,2,5,6,7,8, chromosome complement: 14 cereale, 14 squarrosa.

No. 3, chromosome complement: 13 cereale, 14 squarrosa.

No. 4, chromosome complement: 14 cereale, 13 squarrosa.


1
5705 the squarrosa
Prolific


2
C5842-2


3
1
2
3
4
5
6
7
8

There are only minor variations in the characteristics in most of the plants but in a few instances there are marked differences. In plant height, plants 3 and 4 are shorter than their sibs, plant 2 has a relatively high number of culms, and in plant 4, the average number of spikelets is somewhat lower than the mean. There are marked differences in seed set, plant 4 showing the highest (7.1%) and plant 5 the lowest (0.0%).

The second generation amphidiploids all show a predominance of rye-like characteristics, but there are some marked differences that have been related to aneuploidy. The fertility of the amphidiploids is very low, both in the first and second generations (Table 11). However, the noticeable difference in seed set among the second generation plants is rather interesting. Of the two aneuploids, the one lacking a rye chromosome, plant 3, shows a sharp increase in seed set over all the others. It is possible that the missing chromosome is responsible for one of the aspects of failure in fertilization mentioned earlier.

The second aneuploid, plant 4, which lacks a squarrosa chromosome, set only one seed whereas the remaining plants, although euploid, show a variation in seed set of from 0 to 17 seeds. It may be concluded that the missing squarrosa chromosome does not exert any influence on seed set.

The low seed set in both first and second generation amphidiploids is probably related to more than one source of interference. Amphidiploids with rye as a parent, especially Triticales, are only semi-fertile, a feature that has been attributed by Muntzing (1939) to an automatic degeneration of the rye component as a result of inbreeding enforced on the amphidiploid by the genome of the other parent. Riley and Chapman (1957) on the other hand attribute the infertility in wheat-rye amphidiploids to a general incompatibility between the genotypes of inbreeders and outbreeders. In subsequent studies Riley (1960), by adding individual rye chromosomes to the

wheat complement, was able to demonstrate that each of the 5 different rye chromosomes added had a profound effect in reducing fertility. In his opinion, this effect is of a genetic rather than cytological origin. However, Lamm (1936), Rees (1955) and Sybenga (1958) all report a decrease in chiasma frequency, and subsequently, various degrees of asynapsis, as inbreeding progresses in rye. In view of these reports it appears probable that the reduced fertility observed in the squarrosa-cereale amphidiploids is a result of both genetic intereference with the fertilization process and meiotic instability induced by a reduction in chiasma frequency.

2. Karyotype analysis

The first generation amphidiploid had 28 chromosomes (Plate IX, fig. 1), 14 of which could be identified by chromosome lengths as cereale and 14 as squarrosa chromosomes. These lengths, given in Table 13, are the mean values of the measurements of the 2 homologues. The satellites of Ae. squarrosa are clearly visible but those of S. cereale are not seen. The arm ratios of these chromosomes compare closely with those of the corresponding chromosomes in the F_1 hybrid (Table 10).

In the C_2 generation, 6 of the 8 plants had 28 chromosomes and 2 had 27 as indicated in Table 12. The measurement of the chromosomes in one

TABLE 13

MITOTIC CHROMOSOME MEASUREMENTS OF C_1 AMPHIDIPLOID AE. SQUARROSA X S. CEREALE

	Ae. squarrosa genome					S. cereale genome			
Chrom. no.	Long arms	Short arms	Satellites	Total lengths	Arm ratios	Long arms	Short arms	Total lengths	Arm ratios
I	4.68	3.09		7.77	1.51	5.85	5.53	11.38	1.06
II	4.04	3.30		7.34	1.22	6.17	4.70	10.87	1.31
III	4.26	1.81	.64	6.71	2.35	6.70	3.83	10.53	1.75
IV	3.51	2.98		6.49	1.18	6.17	4.05	10.22	1.52
V	3.62	2.66		6.28	1.36	5.43	4.58	10.01	1.19
VI	3.83	1.97		5.80	1.95	6.60	3.19	9.79	2.07
VII	2.77	2.61		5.38	1.07	5.32	4.42	9.74	1.20

PLATE IX

Figure 1. Somatic metaphase from C_1 amphidiploid Ae. squarrosa x S. cereale.

Figure 2. Meiosis in same amphidiploid.

Figure 3. Somatic metaphase in C_2 amphidiploid, plant no. 3.

Figure 4. Somatic metaphase in C_2 amphidiploid, plant no. 4.

Arrows in figure 2 indicate chromosomes of S. cereale.
The arrow in figures 3 and 4 indicates the monosomic chromosome.

1
2
3
4

cell of each of these aneuploids (not shown) indicates that plant 3 lacked a cereale chromosome (Fig. 2) and plant 4 a squarrosa chromosome (Fig. 3). The arrow in this figure and in Fig. 2 identifies the monosomic chromosome. However, it was not possible to identify these chromosomes as to number in their respective genomes.

3. Meiotic analysis

The analysis of 50 cells of the C_1 plant at metaphase I of meiosis is presented in Table 14. Although each chromosome had an identical homologue, there were only 3 cells in which pairing was complete. The average number of univalents per cell, 3.8 (of which 1.6 were from Aegilops and 2.2 from Secale), indicates an asynaptic effect that appears to have been more pronounced among the rye chromosomes than the Aegilops. Only one heteromorphic bivalent and one trivalent were recorded. The latter is formed by two squarrosa and one cereale chromosomes. According to Gaul and Münzner's formula, the total number of chromosomes potentially capable of pairing in the amphidiploid is 29.2, whereas the observed average bivalent association is 12.14.

This amphidiploid provides a method of checking the accuracy of Gaul and Münzner's formula. Since this is a first generation amphidiploid produced from the F_1 hybrid by means of colchicine, it has 2 identical sets of chromosomes and should, therefore, form 14 bivalents. In other words it should have 28 chromosomes capable of pairing. The estimate given by the formula, 29.2 has an error of 1.2 therefore. This may be due to the fact that the population of cells, 50, was not quite adequate and the resultant extrapolate derived by the formula was exaggerated a little.

TABLE 14

FREQUENCIES AND TYPES OF CHROMOSOMAL ASSOCIATIONS IN *AE. SQUARROSA* X *S. CEREALE* C_1

No. of cells	Univalents		Bivalents			Trivalents
	A	S	AA	SS	AS	AAS
3	0	o	7	7		
10	2	0	6	7		
1	0	1	6	6		1
9	0	2	7	6		
9	2	2	6	6		
3	4	2	5	6		
6	0	4	7	5		
3	2	4	6	5		
1	4	4	5	5		
1	6	4	4	5		
1	3	5	5	4	1	
1	0	6	7	4		
2	4	6	5	4		
Total 50	80	110	310	294	1	1
Av. per cell	1.6	2.2	6.2	5.9	.02	.02

A = *Aegilops*
S = *Secale*

The asynapsis present in the first generation amphidiploid is most probably the cause of its production of aneuploids in the second generation. These aneuploids may provide a method of determining the morphological and genetic effects of individual rye or *Aegilops* chromosomes. O'Mara (1940), Riley (1960) and Evans and Jenkins (1960) have attempted to do this by adding individual rye chromosomes to the wheat complement. Evans and Jenkins found that the individual additions caused only very minor quantitative changes in plant morphology and in the case of rye chromosomes II and III no phenotypic changes were reported. They suggested that the characteristics distinguishing rye from wheat are either hypostatic to the wheat genotype or are not controlled by factors on the single rye chromosome studied. This is rather surprising in view of the considerable effect of the rye genome in combination with the D genome in the *squarrosa-cereale* hybrid. It is certainly to be expected that some rye genes will be dominant or at least will

interact to produce an intermediate effect. More than likely there are morphological differences at the macroscopic level (glume widths and lengths, shoulder types, palea and lemma structure) which will be reported in future papers.

Another approach to this kind of analysis would be to study plants containing an aneuploid number of rye chromosomes close to the diploid number. Monosomics in diploid rye have not been reported, indicating that the diploid system is operating with the minimal amount of genetic material, the loss of a single chromosome leading to gametic or zygotic lethality. However, in the aneuploids derived from the asynaptic 28-chromosome amphiploids, the loss of a pair of the 14 rye chromosomes would be buffered to some extent by the homoeologous pair of squarrosa chromosomes. Consequently, by comparing such aneuploids with their euploid counterparts one might determine (a) the effect of deficiencies of rye chromosomes, (b) the effect of Aegilops chromosomes in the absence of certain rye chromosomes. The great disadvantage at present to this approach is the inability to identify the individual rye chromosome morphologically in the amphidiploid state because of the incorporation of their satellites in the short arms. Nevertheless, the squarrosa chromosomes are readily identified by their total lengths and arm ratios.

III. Ae. cylindrica Host. ssp. pauciaristata x S. cereale L. c v. Prolific

(a) Plant morphology

Ae. cylindrica is an annual with numerous long culms, slightly geniculate-recurved at the base. The spikes are narrow, cylindrical, tapering slightly toward the top. The glumes are 0.6 to 1 cm. long, bidentate at the apex with one tooth triangular, broad, obtuse, and short, and the other one narrow, tapering into an awn or an awn-like appendage. The glumes of the apical spikelet are long-awned and lie between 2 short lateral teeth. The lemmas of the lateral spikelets are bidentate at the apex and the lemma of the apical spikelet is long-awned. The margin of the rachis is denticulate and the spikelets disarticulate readily.

A summary of some of the characteristics of the parents, F_1 hybrid and amphidiploid is presented in Table 15. The hybrid is an annual whose gross morphology approaches that of Ae. cylindrica. It resembles this species in plant height, number of florets per spikelet and in the shape, width and shoulder of the glumes. It is intermediate in spike length and number of spikelets, and the hairy neck character of rye is present. The spikelets are not disarticulate and the margin of the rachis is denticulate. The glume beak extends into an awn 0.3 to 1.0 cm. long on the lower spikelets which is approximately half the length of the lemma awns. The apical spikelet has 4 awns, a short one (0.7 cm.) being present on each glume and a very long one (3.3 to 4.7 cm.) on each lemma. The amphidiploid is similar in appearance to the F_1 hybrid (Plate X, figs. 3,4), and almost identical in morphology (Table 15).

PLATE X

1. Pistillate parent, Aegilops cylindrica Host. ssp. pauciaristata.
2. Staminate parent, Secale cereale L. c.v. Prolific.
3. F_1 hybrid of Ae. cylindrica x S. cereale.
4. C_1 amphidiploid of Ae. cylindrica x S. cereale.


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TABLE 15

COMPARATIVE MORPHOLOGY OF *AE. CYLINDRICA*, *S. CEREALE*, F_1 HYBRID AND C_1 AMPHIDIPLOID

Character	*Ae. cylindrica*	*S. cereale*	F_1 hybrid	C_1 amphidiploid
Plant height	20-50 cm.	90-132 cm.	20 - 53 cm.	25 - 50 cm.
Spike length	7 - 12 cm.	9 - 11 cm.	11 - 16 cm.	11 - 16 cm.
No. of spikelets	6 - 10	28 - 32	12 - 16	10 - 18
Florets per spikelet	3 - 4	2 - 3	3 - 4	3 - 4
Awn length	2.5 - 7 cm.	0.5 - 4.5 cm.	0.5 - 5.0	0.5 - 2.8
Glume shape	oblong	lanceolate	oblong	oblong
" width	medium	narrow	medium	medium
" keel	absent	median, denticulate	sub-median, denticulate	sub-median denticulate
" nerves	7 - 9, denticulate	absent	4-5, denticulate	4-5, denticulate
" shoulder	elevated	wanting	elevated	elevated
" beak	acuminate, short	short, straight, denticulate	acuminate, long	acuminate, long
Hairy neck	absent	present	present	present

Among the 12 characters considered in the hybrid, 7 resemble Ae. cylindrica, 3 resemble S. cereale, one is intermediate and one transgresses the parent. This tendency to resemble the cylindrica parent more than the cereale parent is probably accounted for by the fact that Ae. cylindrica is a tetraploid and its genetic contribution to the hybrid is greater.

Of special interest is the fact that the F_1 hybrid and amphidiploid are awnletted, indicating that Ae. cylindrica contains a gene suppressing the awn development of S. cereale. Ae. cylindrica contains the C genome of Ae. caudata and the D genome of Ae. squarrosa (Matsumoto and Kondo, 1942). It is evident in the hybrid of Ae. squarrosa x S. cereale, which is fully awned, that the D genome does not contain an awn suppressor, and therefore that this gene must be located on a chromosome of the C genome of Ae. cylindrica.

(b) Karyotype analysis

Ae. cylindrica ssp. pauciaristata has 28 chromosomes, one pair of which is satellited. Although detailed measurements of the parental and hybrid chromosomes were not obtained, the somatic metaphases were examined. The parental chromosomes were similar to those described by Chennaveeraiah (1960), one pair having a sub-median to sub-terminal centromere, three pairs with sub-terminal and the remainder with sub-median centromeres. The satellite appeared on the third longest chromosome, designated chromosome III by Chennaveeraiah.

In the hybrid, which had 21 chromosomes, the 14 cylindrica chromosomes appeared similar to the parental chromosomes in morphology. Only one satellited chromosome appeared, which corresponded with chromosome III of Ae. cylindrica. The 7 chromosomes of rye were readily identified by their size (Plate XI, fig. 1), but it was difficult to identify them specifically by number, especially since the incorporation of the satellite into the body

PLATE XI

Figure 1. Somatic metaphase from F_1 hybrid of *Ae. cylindrica* x *S. cereale*.

Figure 2. Meiosis in same hybrid.

Figure 3. Somatic metaphase from F_1 hybrid of *Ae. crassa* x *S. cereale*.

Figure 4. Meiosis in same hybrid.

Arrows indicate chromosomes of *S. cereale*.

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of the chromosomes again occurred as in the hybrids described previously. As a result, these three satellited chromosomes, which were classed as submedian in the parental karyotype, appeared as median in the hybrid karyotype.

(c) Meiotic analysis

Meiosis in the parental species as studied in 75 to 100 pollen mother cells was normal. In the hybrid, the differences in length between the chromosomes of the cereale and cylindrica parents are readily seen (Plate XI, fig. 2). The kinds and frequencies of chromosomal associations in 50 cells are given in Table 16. The greatest part of autosyndesis was due to pairing among the cylindrica chromosomes and only rarely were Secale bivalents seen. However, 10.5 percent of the bivalents formed were due to cylindrica-cereale associations. Trivalents were formed frequently by autosyndesis of the cylindrica chromosomes and with a lower frequency by allosyndesis. Quadrivalents are formed only occasionally by either means. According to Gaul and Münzner's formula, the total number of chromosomes potentially capable of pairing is 14.4. A meiotic analysis of the amphidiploid is not included in this study.

TABLE 16

FREQUENCIES AND TYPES OF CHROMOSOMAL ASSOCIATIONS IN AE. CYLINDRICA X S. CEREALE F_1

	Bivalents			Trivalents		Quadrivalents		
	AA	SS	AS	AAA	AAS	AAAA	AAAS	AASS
Total	153	1	15	44	7	2	1	1
% of total	90.5	0.6	8.8	86.2	13.6	50	25	25
Av. per cell	3.1	.02	.30	.88	.14	.04	.02	.02

Av. biv. assoc. per cell: 4.56

The metaphase pairing in this hybrid is different from that of the hybrids involving Ae. comosa and Ae. squarrosa described earlier, in the fact that the majority of associations, both bivalent and multivalent, are formed by cylindrica chromosomes alone. The greatly increased pairing of the Aegilops chromosomes in this hybrid as compared with the previous ones is explained by the fact that Ae. cylindrica is a tetraploid containing the C genome of Ae. caudata and the D genome of Ae. squarrosa. The artificial hybrids between these two species shows an average of 4.74 bivalent associations (Sears, 1941); therefore an average frequency of such associations may be expected through autosyndesis of the cylindrica chromosomes. Indeed, by omitting consideration of the intergeneric associations, an average bivalent association value of 4.20 is found.

The pairing potential value of 14.4 suggests that at least 7 bivalents may be formed through intergeneric and intrageneric associations. It is difficult to determine the validity of this statistic however, in view of the limited number of cells that were analyzed.

IV. Ae. crassa Boiss. ssp. Vavilovii Zhuk. x S. cereale L. c v. Prolific

(a) Plant morphology

The hexaploid subspecies of Ae. crassa is an annual, more or less geniculate-recurved at the base. The spikes are long, thick and brittle and are up to 0.7 cm. wide, somewhat bead-like and covered with appressed hairs. The spikelets are cylindrical and slightly broadened at the base. Rudimentary spikelets are occasionally present. The lemmas of lower spikelets have short awns (1.3 cm.), those of upper spikelets have longer ones (5.0 cm.) and the awns are often recurved.

The hybrid is an erect annual with many culms, whose general appearance resembles the female parent Ae. crassa. The spikes are as long as those of Ae. crassa but are a little slimmer. The spikelets are less rounded, less brittle and less pubescent, and taper a little at the base. Rudimentary spikelets are sometimes present. The lemmas of lower spikelets have short awns (1.1 cm.), those of upper spikelets have longer ones (4.0 cm.) and the awns are straight. The glumes are slightly tapered toward the apex and terminate in an elongated beak and an apiculate shoulder. A summary of some of the chracteristics of the parents and hybrid are given in Table 17. Spikes of the parents and hybrid are shown in Plate XII, figs. 1-3.

TABLE 17

COMPARATIVE MORPHOLOGY OF AE. CRASSA, S. CEREALE AND THEIR HYBRID

Character	Ae. crassa	S. cereale	F_1 hybrid
Plant height	20 - 45 cm.	90 - 132 cm.	30 - 56 cm.
Spike length	10 - 15 cm.	9 - 11 cm.	9 - 16 cm.
No. of spikelets	5 - 12	28 - 32	8 - 17
Florets per spikelets	3 - 4	2 - 3	3 - 4
Awn length	1.3 - 5.0 cm.	0.5 - 4.5 cm.	1.1 - 4.0 cm.
Glume shape	oblong	lanceolate	rectangular
" width	0.4 - 0.5 cm.	0.1 cm.	0.4 - 0.5 cm.
" length	0.7 - 1.0 cm.	0.9 - 1.1 cm.	0.9 - 1.1 cm.
" keel	absent	median, denticulate	absent
" nerves	5 - 9	absent	5 - 9
" shoulders	square to round	absent	apiculate
" beak	short, denticulate	short, denticulate	elongate, denticulate
Hairy neck	absent	present	present

PLATE XII

1. Pistillate parent, Aegilops crassa Boiss. ssp. Vavilovii Zhuk.
2. Staminate parent Secale cereale L. c.v. Prolific.
3. F_1 hybrid of Ae. crassa x S. cereale.


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Of the 13 characters recorded in the present study, 6 resemble Ae. crassa, one S. cereale, 3 are intermediate, 2 are transgressive and one appears to be new. The transgressive characters are spike length and beak length. The new character is the form of the glume shoulder. The shoulder of Ae. crassa is square to rounded but it is absent in S. cereale. Unexpectedly, the shoulder in the hybrid is apiculate.

Zennyozi (1959) in his study of a crassa-rye hybrid also reported transgression in the hybrid spike and the presence of a majority of crassa characteristics. Other characteristics were intermediate and the only rye trait present was the hairy neck, according to his studies.

Again the predominant influence of the Aegilops parent in the hybrid is probably due to its contribution of 21 chromosomes as compared with 7 from Secale. Although the hybrid is distinctly different from Ae. crassa, it is nevertheless closer to it than to the rye parent. The influence of the rye chromosomes is surprisingly slight, the only character identified positively as belonging to rye being the hairy neck.

Apparently an awn suppressor gene is also present in one of the genomes of Ae. crassa. Kihara (1959) proposed that the genome constitution of Ae. crassa is DDM^{cr} but Chennaveeraiah (1960) expressed some doubts regarding this. If Kihara is correct, then this gene is present in the M genome of Ae. crassa, since it has been shown that it is not present in the D genome.

(b) Karyotype analysis

The 2n chromosome number of Ae. crassa Boiss. ssp. Vavilovii was found to be 42, and thus is in agreement with counts by other workers in this field. Pathak (1940), Senjaninova-Korczagina (1932) and Chennaveeraiah (1960) showed three pairs of satellites which correspond with those seen in this study. Three pairs of chromosomes have median centromeres and the

remainder have sub-median ones. A somatic cell of the hybrid (2n = 28) is shown in Plate XI, fig. 3. The satellites of the crassa chromosomes are clearly seen but those of Secale are not. The failure of the rye satellites to appear, again suggesting a satellite dominance as in the previous three hybrids, is perhaps in accordance with the fact that the genomes present in these two hybrids, the D and M, are also present in Ae. crassa x S. cereale.

Measurements of the parental and hybrid chromosomes were not obtained for this experiment. However the chromosomes of the hybrid could be readily separated into their component parental genomes. The assumption was made that the size differences between the chromosomes of Secale and Aegilops are relatively the same in this cross as in the crosses involving Ae. squarrosa and Ae. comosa. This assumption appeared to be justified in view of the fact that Ae. crassa contained the genomes of these two species. Furthermore, the karyotypes of 25 cells contain 7 large and 21 smaller chromosomes, the larger ones resembling the haploid set of Secale with the exception of 3 that failed to reveal their satellites and the 21 smaller chromosomes generally resembling the haploid set of Ae. crassa.

(c) Meiotic analysis

Meiosis in the parental species as studied in 50 cells was relatively normal. In Ae. crassa, 4-6 open bivalents were frequently seen but no other irregularities appeared. In the hybrid the differences in size between the cereale and crassa chromosomes were maintained, but somewhat more difficulty in distinguishing the rye chromosomes from the crassa group was encountered. However, by selecting only the cells with well-spread chromosomes and by relating the number of remaining univalents to the number of chromosomes participating in pairing, it was possible to analyze this hybrid in a quantitative manner.

It will be noted that the average number of bivalents per cell is

4.94. This value is in very close agreement with the results obtained by Zennyozi (1959), who, although unable to distinguish the parental chromosomes in his crassa - rye hybrid, reported a mean of 4.98 bivalents.

The occurrence of autosyndesis of the crassa chromosomes to the extent of 93 percent of the total bivalents, (Table 18), is direct evidence of preferential pairing. In the diploid Aegilops x Secale hybrids, autosyndesis of the Aegilops chromosomes was 11.6 percent for the comosa chromosomes and 12.2 percent for the squarrosa chromosomes. In the hybrid involving tetraploid Ae. cylindrica autosyndesis of the Aegilops chromosomes jumped to 90.5 percent.

TABLE 18

FREQUENCY OF CHROMOSOMAL ASSOCIATIONS IN THE F_1 HYBRID AE. CRASSA X S. CEREALE

No. of cells	Univalents		Bivalents			Trivalents	
	A	S	AA	SS	AS	AAA	AAS
1	12	5	3		1		1
1	12	5	3	1		1	
2	13	5	4	1			
1	14	5	2	1		1	
1	15	5	3	1			
1	16	5	1	1		1	
1	7	6	5		1	1	
1	7	6	6				1
2	9	6	5				1
1	10	6	5		1		
1	12	6	4		1		
1	7	7	4			2	
1	7	7	7				
2	8	7	5			1	
2	9	7	6				
2	10	7	4			1	
2	11	7	5				
5	12	7	3			1	
3	13	7	4				
1	14	7	2			1	
1	15	7	3				
Total 33	367	211	133	6	4	16	4
Average per cell:	11.1	6.4	4.0	.18	.12	.47	.12

Av. biv. assoc. per cell: 4.94

Calculating the average autosyndetic bivalent frequency from the numbers of bivalents and multivalents entirely of Aegilops origin gives a value of 4.6. This value is much lower than would be expected according to Kihara's suggestion that the genomic formula for Ae. crassa is DDM^{cr}, since the presence of the D genomes would provide for perfect autosyndetic pairing of the 7 chromosomes of the genome. It would therefore appear probable either that considerable differentiation of the two D genomes in Ae. crassa has occurred, or that one of them is not a D genome. It would also appear probable, in view of the above data, that the M^{cr} genome is not homoeologous with the D genome, and, indeed, that there is only slight homoeology between any of the three genomes of this species. Therefore, this study supports Chennaveeraiah's opinion that the third genome of Ae. crassa may not be a D, but it also considers his suggestion that it is related to the M^{cr} genome open to question for similar reasons.

The value for the number of chromosomes potentially capable of pairing is 17.8 or a total of 8.9 bivalents. However, due to the low number of cells analyzed, this statistic may not be reliable. As Gaul (1958) indicated, one of the requirements for the use of the formula is a sufficiently large population of cells.

GENERAL DISCUSSION

1. Nature of pairing

Conclusions derived from the evidence which establishes the fact that the pairing of Aegilops and Secale chromosomes is due to genetically homologous segments are: (a) Chiasmata were formed frequently indicating that the areas involved were not minute relics of ancestral homologies but were segments of such magnitude as to permit frequent crossing over; (b) Potential Aegilops-Secale pairing was rather high, especially in the hybrids involving diploid Ae. squarrosa and Ae. comosa. If the maximum amount of pairing may be used as an indication of the pairing potentiality, the cell in the squarrosa-cereale hybrid (Plate VII, fig. 9) is convincing evidence that the intergeneric homologies are extensive.

Non-homologous pairing has been discussed by McClintock (1933) in Zea, Lammerts (1934) in polyhaploids in Nicotiana and Levan (1942) in Secale. McClintock suggested that non-homologous pairing may occur as a result of the torsion that determines relational coiling. Levan furthermore believed that such pairing could lead to chiasma formation. However, as Darlington pointed out, the evidence shows that none of these kinds of torsion pairing results in crossing-over and chiasma formation. Furthermore where there is non-homologous torsion-pairing, it is impossible to prove the complete absence of homologous attraction-pairing. On the other hand, Kostoff (1938) showed that complete homology is not necessary for synapsis and crossing-over. Where homoeology is the result of translocations the homologous segments are still capable of synapsis and chiasma formation. Beadle (1932) proved that crossing-over and chiasma formation occurred between chromo-

somes of different genera. By using genetic markers and a translocation as a cytological marker, he showed that crossing-over between the long arm of chromosome 9 of Zea Mays and one from Euchlaena mexicana occurs with almost the same frequency as in maize. Similarly Maguire (1960) found that intergeneric pairing occurred with a frequency of 93 percent in Zea-Tripsacum segments.

Where chromosomes differ markedly in size between species, these chromosomes are generally suspected of being non-homologous. Several reports dealing with this aspect indicate that homology as evidenced by chromosome pairing is not precluded by differences in chromosome size. Hakansson (1934) noted that the chromosomes of Godetia deflexa are one-third to one-quarter as long as those of G. Bottae and that this size differential is maintained in the hybrid during metaphase both in somatic and meiotic cells. As a result, during meiosis in the hybrid, heteromorphic bivalents are formed. The fact that these chromosomes are from the same genera lends support to the view that homology between them exists and that homology and chromosome size are not always directly related.

Another report showing a higher degree of heteromorphic bivalent formation was presented by Endrizzi and Phillips (1960). The chromosomes of Gossypium arboreum are larger than those of G. raimondii and the hybrid between them showed an average of 5.9 and a maximum of 12 out of the possible 13 bivalents. The interesting feature here is that all of the bivalents except 5 in the 269 studied were heteromorphic thus indicating that allosyndetic attractions were stronger than autosyndetic ones and that again, chromosome size and homology are not always directly related.

A classical example of homologous pairing between chromosomes from different genera is that reported by Peto (1933) in hybrids between Festuca pratensis and Lolium perenne. Morphologically these two diploid species are

strikingly different and have been widely separated taxonomically. The former has a panicle inflorescence whereas the latter has a spike, indicating considerable morphological divergence. Cytologically, however, there appears to be very little chromosomal divergence since their hybrid showed 7 bivalents at meiosis. This example emphasizes one of the difficulties encountered in correlating morphological and cytological evidence in taxonomy: morphological divergence is not always paralleled by cytological divergence. It is not suggested, on the other hand, that the perfect pairing cited above indicates complete genetic homology. Certainly many genic and perhaps minor structural differences exist between Festuca and Lolium, but evidently there are homologous segments on their respective chromosomes which are of sufficient magnitude to permit prophase pairing and chiasma formation.

The work of Zinder (1960) has shown that extensive linear series of genes may be identical in both nature and position in the widely separated genera Salmonella and Escherichia. In view of this and of the high probability of similarity between large numbers of essential genes in Aegilops and Secale, it would appear likely that the reduced pairing of chromosomes is an aspect of genes modifying pairing or of relatively gross structural re-arrangements. It is rather unlikely that such re-arrangements are the determining factor in the diploid Aegilops-Secale hybrids in view of the very low multivalent formation between the chromosomes of these two genera.

2. The pairing modifier

It appears highly probable that some mechanism is present in the Aegilops-Secale hybrids that modifies the pairing of homoeologous chromosomes in a manner similar to the pairing restrictor described by Riley et al and Sears and Okamoto (Cf. rev. lit.). Although the phenomenon has not been

studied in the detail as that in Riley's material, several reasons may be advanced for its presence. First, the homologous regions between the Aegilops and Secale chromosomes in those crosses studied appear to be extensive in view of the high frequency of chiasmata and their complete terminalization in heteromorphic bivalents. This is reflected in the high potential pairing values obtained. Secondly, the actual pairing frequency is much lower than that expected according to the above criteria of homology. Thirdly, Aegilops longissima, Ae. bicornis, and Ae. sharonensis have been shown (Riley et al 1958) to react with the genus Triticum in the same way.

The hypotheses advanced at this time are that: (1) the relative chromosomes of Aegilops and Secale have more than a residual homology; (2) the length of these homologous regions are of sufficient magnitude to permit prophase pairing and chiasma formation; (3) a genetic mechanism exists which modifies such pairing and chiasma formation.

This mechanism appears to be general to the Aegilops-Secale hybrids studied here, but the effect is not uniform in all as seen in Table 19.

TABLE 19

COMPARISON OF OBSERVED AND POTENTIAL BIVALENT FREQUENCIES# IN HYBRIDS OF SECALE WITH AE. COMOSA, AE. SQUARROSA, AE. CYLINDRICA AND AE. CRASSA

Hybrid	Observed bivalents	Potential bivalents	Observed A-S*	Potential A-S*
Ae. comosa x S. cereale	0.48	3.3	.38	2.50
Ae. squarrosa x S. cereale	1.12	6.3	.70	3.90
Ae. cylindrica x S. cereale	4.56	8.9	.24	0.44
Ae. crassa x S. cereale	4.94	8.9	.24	0.44

Mean frequency per cell
* A-S : Aegilops-Secale associations

Since the observed bivalent frequency (OBF) and the observed A-S frequency (OA-S) are available from the data and the potential bivalent frequency (PBF) is calculated from Gaul and Munzner's formula, it is possible to derive the potential A-S frequency (PA-S) from the ratio $\frac{OA\text{-}S}{OBF} = \frac{PA\text{-}S}{PBF}$. Thus the observed bivalent frequency in the squarrosa (D genome) hybrid is approximately twice that of the comosa (M) hybrid indicating perhaps that the parents differ structurally as well as genically. Similarly the potential bivalent frequency is almost double. The number of A-S bivalents both observed and potential are similarly greater. Although the intergeneric pairing potential in the comosa hybrid is lower than that in the squarrosa one, it is nevertheless higher than would be anticipated on the basis of taxonomic classification. The rather high pairing potential observed in the squarrosa hybrid in addition strongly indicates that a pairing modifier is present which reduces the pairing of homologous chromosomes. In the tetraploid cylindrica (CD) hybrid, the observed bivalent frequency increased with the additional C genome but not as much as it was potentially capable. The potential number of A-S bivalents is only slightly greater than the observed number in this hybrid but it is down sharply from that of the previous two hybrids. This reduction is largely due to autosyndetic pairing of the C and D genomes which involved some of the chromosomes homologous with those of rye. The observed bivalent frequency of the crassa (DDM^{cr}) hybrid is only slightly higher than that of the cylindrica hybrid despite the presence of a third Aegilops genome. Considering the relatively high potential bivalent frequency and the fact that intergeneric pairing of chromosomes is very low in this hybrid, several inferences can be made. First, the low pairing frequencies indicate that the third genome in Ae. crassa is not another D as suggested by Kihara. Alternatively, if it is a D genome, it must be differentiated to the extent that the homologies with the other

D genome are reduced as well as those with the M^{cr}. Thirdly, if it is a modified D genome, a pairing restrictor may be present which limits homoeologous pairing. In view of the preliminary nature of the study with polyploid Aegilops more evidence must be available before accurate conclusions may be drawn.

A consideration of some of the literature as it supports the idea of a pairing modifier may promote a clearer understanding of the problem. Numerous authors have expressed the opinion that a gene or genes control chromosome pairing. Those concerned with asynapsis or desynapsis of completely homologous chromosomes have been mentioned but are probably related to different mechanisms from that under consideration at present. However it isn't the lack of pairing alone which suggests the presence of a pairing modifier, but the occurrence of hybrids which show a marked increase in pairing following a period of asynapsis. An example of this is given by Ray and Chisaki (1957) in studies on Amsinckia. Individuals were found intermediate in phenotype between A. intermedia and A. Menziesii some of which showed extremely irregular meiosis and others which showed normal meiosis. They suggested that this population represented an instance of natural hybridization between these two species followed by selfing of the progeny and segregation of a variety of forms in F_2. It would appear that one of the factors involved in this segregation was a pairing modifier.

Another example is given by Nielsen (1956) in a naturally occurring Agropyron x Elymus hybrid. The derivatives of this hybrid segregated for Agropyron and Elymus characters but were relatively uniform in the regularity of meiosis, the most common configuration being 14 bivalents. He suggested that the original hybrid was sterile and was backcrossed to the Elymus parent with successive generations recovering their fertility. Here again the inference may be drawn that the pairing modifier which was present in the early generations was eliminated in the segregates.

An absence or suppression of the pairing modifier is suggested in hybrids with a high degree of pairing where one would expect little or no pairing on the basis of pronounced morphological and taxonomical divergence of the parents. Although no suppressors of this kind were encountered in the present study, other reports suggest that such a mechanism may be present in some hybrids of remote origin. For example, most of the Festuca-Lolium hybrids reported by Peto exhibited normal pairing, indicating that one of the parents, probably Festuca, contains a gene or genes which suppress the action of a pairing modifier, enabling the homoeologous chromosomes to synapse and form bivalents regularly. One-quarter of the backcrosses to Lolium were asynaptic indicating that Lolium contains the pairing modifier which limits such homoeologous associations. A suppressor such as that in Festuca has been reported by Riley et al (c.f. rev. lit.) in Ae. speltoides which inhibits the action of the pairing restrictor on chromosome V. In effect this mechanism is a pairing inducer.

Whenever fertility is encountered in an F_1 hybrid between distantly related parents, relatively regular meiotic pairing is indicated and, probably, a gene inducing such pairing is involved in suppressing the mechanism which restricts associations of homoeologous chromosomes. Such a hybrid was reported by Rick (1960) involving Lycopersicon esculentum, the garden tomato, and Solanum pennellii, a nightshade from Peru which was recently named as a new species. In common with the parents, this hybrid contained 12 bivalents at metaphase though 20 percent of the cells had a quadrivalent. Four of the bivalents were heteromorphic according to Rick but no indication was given of their generic origin. Another intergeneric hybrid which provides evidence in support of the theory of a pairing inducer is Nakajima's Secale x Haynaldia cross (c.f. rev. lit.) showing partial

fertility in the first genera tion. Similarly Ono (1943) and Ono and Sato (1935) reported fertility in first generation hybrids of Paraixeris x Lactuca and Paraixeris x Crepidiastrum (cf. rev. lit.).

It is apparent, therefore, that the concept involved in the modification of chromosome pairing in hybrids is not to be explained by the effects of a single gene but by those of a complex of factors. Much additional information is required in order to determine: (1) the extent to which pairing restrictors and inducers occur in other species, if at all; (2) whether such mechanisms are specific in their action (i.e. do they perform similarly in all hybrid combinations); (3) what is their fate in polyploids if they are present in all diploids (i.e. dominance relationships).

CONCLUSIONS

The foregoing observations, considered in the light of other studies have led to several conclusions regarding the cytology, morphology and intergeneric relationships of Aegilops, Secale and their hybrids. Morphologically the hybrids tended to resemble the parent with the higher chromosome number, but at the diploid level, the two hybrids differed markedly in this respect. The hybrid Ae. squarrosa x S. cereale strongly resembled the rye parent, whereas the hybrid Ae. comosa x S. cereale bore a stronger resemblance to the Aegilops parent indicating that Ae. comosa contains a greater number of dominant genes than Ae. squarrosa in relation to S. cereale. The hybrid Ae. cylindrica x S. cereale contained only a few visible rye traits,whereas Ae. crassa x S. cereale had only one rye characteristic, the remainder being either intermediate or of Aegilops origin. Awn inhibition was noted in the hybrids involving Ae. comosa, Ae. cylindrica and Ae. crassa but not in Ae. squarrosa, suggesting that the M and C genomes contain a gene suppressing awn development in the hybrid.

The somatic chromosomes of Ae. squarrosa, Ae. comosa and S. cereale are relatively uniform in length within their own genomes, but can be distinguished from each other by their absolute lengths, relative lengths, arm ratios and the presence of characteristic satellites. The somatic chromosomes of S. cereale are larger than those of Aegilops and this size difference is maintained in the meiotic and somatic cells of their hybrids. In the latter, a competitive relationship of satellites between the two genera is suggested by the failure of the three satellites of Secale to appear. The Secale chromosomes can therefore only be distinguished as a group in these hybrids.

Because of the size-differential of chromosomes in the meiotic cells of the hybrids, it was possible to identify the Secale and Aegilops chromosomes which participated in pairing. The most significant finding was that pairing occurred between the chromosomes of Aegilops and Secale in all of the hybrids

studied. The extent of this allosyndetic pairing was lower in the comosa than the squarrosa hybrid. It was suggested that the pairing observed between the chromosomes of diploid Aegilops and Secale was much lower than their maximum pairing potential and that this pairing was under genetic control.

In the amphidiploid Ae. squarrosa x S. cereale, asynapsis of some Secale and Aegilops chromosomes led to aneuploid progeny. These aneuploids, one lacking a Secale chromosome resulting in increased fertility and the other an Aegilops chromosome resulting in reduction in plant height, suggested a method of chromosome analysis which related morphology with cytology.

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